(54) Title: NEMATODE MODEL FOR ALZHEIMER'S DISEASE

(57) Abstract

Provided are worms having deletions in genes associated with Alzheimer's disease and methods for detecting genes and compounds involved in the causation of Alzheimer's disease or useful for the treatment of Alzheimer's disease. A novel method for constructing deletions in the nematode is also provided.

NEMATODE MODEL FOR ALZHEIMER'S DISEASE

Background of the Invention

5   The field of the invention is Alzheimer's disease.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder of the central nervous system involving loss of memory and cognitive function. Neuropathological hallmarks of AD extracellular amyloid plaques, whose major component is the Aβ peptide, a proteolytic product of the β-

10   amyloid precursor protein (APP).

Dominant mutations in any of three mammalian genes, *PS1*, *PS2*, and *APP* are causative for the early-onset, familial form of AD (FAD). *PS1* and *PS2* encode related proteins-, while *APP* encodes the amyloid precursor protein (APP) (reviewed in Hardy, Trends Neurosci. 20:154-159, 1997), from

15   which the Aβ peptide is generated by proteolytic processing.

Since presenilin-1 (PS1) and presenilin-2 (PS2) were first identified as genes mutated in FAD, much effort has been devoted to elucidating the biological function of presenilins in normal and disease states. Two lines of evidence support a hypothesis that presenilins affect proteolytic processing of

20   APP First, PS(FAD) mutations are associated with an increase in a longer, more amyloidogenic form of Aβ known as Aβ42 ; and second, levels of Aβ are decreased several-fold in neurons derived from PS1 knockout mice relative to PS1(+) control mice. Moreover, in neurons, Aβ 42 may be generated in the endoplasmic reticulum, where presenilins have been localized .

25   *C. elegans* has three presenilin genes, *spe-4* (spermatogenesis defective) (L'Hernault, et al., J. Cell Biol., 119:55-68, 1992), *sel-12* (suppressor and/or enhancer of *lin-12*) (Levitan et al., Nature 377:351-354, 1995), and *hop-1* (homolog of presenilin) (Li et al., Proc. Natl. Acad. Sci USA 94:12204-

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12209, 1997). Rescue experiments using transgenes have shown that human PS1 and PS2 can substitute for SEL-12, demonstrating that presenilin function has been conserved from nematodes to mammals.

Construction of a nematode having mutations in nematode presenilin genes and also having phenotypes useful for screens for genes associated with the biological pathway and for screens for compounds affecting the biological pathway in which these genes function would be useful for identifying therapeutics and causative agents involved in AD.

Summary of the Invention

We have discovered an improved nematode model for studying Alzheimer's disease and for screening for Alzheimer's therapeutics. Also provided is a novel method for reverse genetics.

In a first aspect, the invention features an isolated nucleic acid encoding a HOP-1 polypeptide having a mutation (preferably a deletion in or including the *hop-1* gene), relative to a Hop-1 encoded by the wild-type *hop-1* gene. In preferred embodiments the nucleic acid is cDNA, genomic DNA, or RNA and the mutation is a deletion.

In a third aspect, the invention features a nematode having an engineered mutation in the *hop-1* gene, preferably a deletion. More preferably, the *hop-1* nematode further includes a deletion or other null mutation in *sel-12* or a deletion in *spe-4*. More preferably, the nematode has deletions or other null mutations in *hop-1*, *sel-12*, and *spe-4*. In a preferred embodiment, the nematode is homozygous for at least one of the aforementioned mutations.

In a fourth aspect, the invention features a method for identifying a compound capable of ameliorating diseases, including Alzheimer's disease.

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The method includes exposing a nematode having a *hop-1* deletion or other

mutation and a second deletion or other mutations in a gene selected from the

group consisting of *sel-12* and *spe-4* to an effective amount of a compound and

screening for an alteration in the phenotype of said nematode.   Examples of

5   such alterations are described herein below. An alteration in the phenotype of

the nematode indicates a compound capable of ameliorating disease.

Preferably, the phenotype is a phenotype conferred by the presence of *hop-1*,

sel-12, *spe-4*, or the combination thereof, present in the nematode being used in

the screen. In a preferred embodiment of this method the second mutation is a

10   mutation in *sel-12*, the phenotype is selected from the group consisting of dead

eggs or sterility, and the alteration is an alteration in either the number viable

eggs or fertile offspring where such eggs or offspring, would otherwise be

absent. Additional phenotypes which may be screened for to detect compounds

which positively or negatively affect the genesis and progression of AD are

15   also a part of the invention. Such screens identify both potential prophylactics

and therapeutics for AD and environmental agents which may exacerbate the

disease. In some of these embodiments of the invention wild-type worms or

single mutations may be used (see the Detailed Description, below).

In a fifth aspect, the invention provides a method for identifying a

20   gene involved in the development or amelioration of disease, e.g., Alzheimer's

disease. The method includes selecting or screening for a reversion mutation

(suppressor mutation) in a nematode having a *hop-1* deletion and at least one

additional second mutation in a gene selected from the group consisting of *sel-*

12 and *spe-4*, and screening for an alteration in the phenotype of said

25   nematode, an alteration in the phenotype of said nematode indicating a

compound capable of ameliorating the disease. In preferred embodiments the

second deletion is a deletion in *sel-12* or *sel-12* and *spe-4*, the phenotype is

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selected from the group consisting of dead eggs or sterility and those phenotypes provided herein, and the alteration is an alteration either of viable eggs or fertile offspring where such eggs or offspring, would otherwise be absent or one of the additional alterations provided herein. In a preferred

5   embodiment, the method includes the additional step of screening for suppressors of the suppressor genes identified in the first selection or screen. In this embodiment, the suppressor gene is preferably placed in a genetic background in which it has a discernable phenotype.

In a related aspect, the invention provides a method for screening for

10   genes associated with AD wherein the starting strains lack mutations in *spe-4*, *hop-1*, or *sel-12*. In this aspect the method includes looking for those phenotypes identified herein to be associated with the *hop-1; spe-4* and *hop-1; sel-12* worms or the *hop-1; spe-4; sel-12* triple mutant.

In the seventh aspect, the invention features a method for

15   constructing a nematode having a genetic deletion in a region of choice. The method includes the steps of: a) exposing said nematode to an appropriate mutagen; b) propagating said nematode for at least one generation; c) amplifying the nucleic acids of said nematode using at least two nucleic acid primers from the region of choice in the genome; and d) detecting the presence

20   of a amplified nucleic acid which is shorter than the amplified nucleic acid from a non-mutagenized nematode. In preferred embodiments, the mutagen is selected from the group consisting of ethyl methanesulfonate, nitrosoguanidine, diethyl sulfate, *N*-nitroso-*N*-ethylurea, acetaldehyde, diepoxyoctane, diepoxybutane, trimethylpsoralen followed by UV irradiation, ^{32}P decay, and

25   ionizing particles. In another embodiment the mutagen is γ-irradiation, ultraviolet irradiation, or X-irradiation.

In various other preferred embodiments the nematode is *C. elegans*;

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the method further comprises the steps of initially amplifying nucleic acid from a pool of nematodes said pool comprising at least 1000 genomes derived from distinct mutagenized nematodes; the primers have the sequences of nucleic acids within 10 kilobases of said region of choice (more preferably within 5 kilobases of said region); the nematode exposed to said mutagen is a part of a pool of at least 1000 nematodes; the detecting includes at least two rounds of amplifying, the first round of said amplifying being amplifying of nucleic acid using nucleic acid isolated from a pool of nematodes, said pool including at least 1000 nematodes genomes from the original mutagenesis.

In another preferred embodiment, the nematode being mutagenized has a genetic marker within 5 map units of the nucleic acids where said genetic alteration is desirable or, even more preferably has a second genetic markers within 5 map units of the first genetic marker, the second genetic marker being on the opposite side of the nucleic acids in the region of choice, relative to the first genetic marker. Where genetic markers are used they may be dominant or recessive.

Preferred primers have sequences which are within 5 kilobases of each other in a non-mutagenized nematode genome.

By "deletion" is meant removal of at least 1 nucleotide from the target region of the genome. Preferably, the number of nucleotides removed is between 200 and 5000 and, most preferably, between 500 and 3000. Most preferably, the deletion is sufficient to reduce or eliminate a biological function of a protein encoded by the nucleic acid region of choice. "Deletion" as used herein in the context limited to *hop-1, spe-4*, and *sel-12* may also include null mutations other than deletions. Preferably, such null mutations have a low frequency of reversion.

By "nematode" is meant any nematode of the class Secernentea.

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useful for genetic research. For example, *C. elegans*, *C. briggsae*, and *C. vulgaris* may be used. Most preferably, the nematode is *C. elegans*.

By "region of choice" is meant any nucleic acid region in the nematode where a deletion is desired. For example, a nucleic acid region that is capable encoding for a polypeptide sequence, capable of regulating gene expression, capable of regulating RNA stability, or capable of altering chromosome structure are all included.

By "Bag of Dead Eggs" means a phenotype which is the phenotype observed in *hop-1;sel-12* and *hop-1/sel-12/+* progeny animals segregating from a *hop-1/+;sel-12/+* or *hop-1/+; sel-12*. The phenotypes include homozygotes that have a combined maternal-effect embryonic lethality and Egl phenotype.

Brief Description of the Drawing

Fig. 1A-1B show schematic representations of the *hop-1* and *sel-12* loci in wild-type (+) and deletion -containing animals. Black boxes represent coding sequences, open boxes represent 3' untranslated sequences and lines represent extragenic sequences (Levitan et al., Nature 377:351-354, 1995; Li et al., Proc. Natl. Acad. Sci. USA 94:12204-12209, 1997). A) The extent of the *nr2003* deletion is indicated by the gap. F1, F2, R1, and R2 denote the approximate locations of the nested PCR primers used to identify the *nr2003* deletion. B) The extent of the *nr2001* deletion is indicated by the gap. Primers used to identify the *nr2001* deletion are designated as in Fig. 1A.

Figs. 2A-2D show the germ line and somatic gonads of *hop-1;sel-12* animals display Glp-1 and Lin-12-like phenotypes. Shown are differential interference contrast (DIC) photomicrographs of wild-type (Figs. 2A and 2C) or strain A *hop-1;sel-12* (Figs. 2B and 2D) hermaphrodites. Fig. 2A) A gonad arm of a young adult hermaphrodite containing undifferentiated germ cells (gc)

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near the distal tip cell (dtc) and oocytes (ooc) and sperm (sp) proximally. Fig.
2B) A Glp-1-like (Austin et al., Cell 51:589-599, 1987) gonad arm containing
sperm (sp) distally as well as proximally, but no oocytes or undifferentiated
germ cells. This field of view also shows a protruding vulva (Ppvul). Fig. 2C)

5   An L4 hermaphrodite containing a single anchor cell (AC). Fig. 2D) An L4
hermaphrodite containing two Acs. Scale bars, 10 μm.

Figs. 3A-3F show strain B *hop-1;sel-12* embryos exhibit defects
similar to those observed in a conditional *glp-1(lf)* mutant. (Figs. 3A-3C)
Immunofluorescence micrographs of embryos stained with the monoclonal

10   antibody J126 in order to visualize the intestinal valve cells (Mango et al.,
Development 120:2305-2315 1994). Fig. 3A The arrow points to a pair of
intenstinal valve cells in a wild-type embryo. Neither *glp-1* (Mello et al., Cell
77:95-106 1994) Fig. 3B nor *hop-1;sel-12* Fig. 3C mutant embryos exhibit
intestinal valve cell staining. The embryo in the Fig. 3C mutant embryos

15   exhibit intestinal valve cell staining. The embryo in Fig. 3C is representative
os 25 embryos scored. Pharyngeal gland cells also stain with the J126 antibody
(Mango et al., Development 120:2305-2315, 1994). Pharyngeal gland cell
staining is visible in (Figs. 3A-3C); in Fig. 3A most of the staining is in a
different focal plane than that shown. Figs. 3D-3F show immunofluorescence

20   micrographs of embryof stained with the monoclonal antibody 9.2.1, which
recognizes pharyngeal myosin C (Epstein et al., in Muscle Development, eds.
Pearson & Epstein, Cold Spring Harbor Lab., Cold Spring Harbor, NY pp. 7-
14, 1982). *glp-1* (Priess et al., Cell 51:601-611 1987) (Fig. 3E) and *hop-1;sel-
12* (Fig. 3F) embryos have less pharyngeal tissue than a wild-type embryo (Fig.

25   3D). The embryo depicted in (Fig. 3F) is representative of more than 50
embryos scored. *C. elegans* embryos are approximately 50 μm in length.

Fig. 4 shows a schematic representation of the wild type R03E1.akt

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gene and the identified deletion. Boxes represent predicted exons and lines between boxes represent introns. The approximate locations of the PCR primers used in the screens are indicated by horizontal short bars and primer names. The approximate location of the deletion is indicated by the gap. The

5   two vertical short bars represent possible deletion end points.

Fig. 5 shows gel pictures of samples from an initial PCR screen of *C. elegans* mutagenized with ethyl nitroso urea (ENU). Pools of worm lysates were screened by nested PCR using primers *sel-12.F1*, *sel-12.F2*, *sel-1.R1* and *sel-1.R2*. Two independent sets of DNA were assayed for each pool. The

10   wild-type 2.7 kb product is seen in every lane. Pool #5, set 2, also shows a potential 1.3 kb deletion (GIBCO-BRL 1 kb ladder DNA molecular wight standards).

Fig. 6 shows a gel demonstrating that worms containing deleted DNA were recovered from frozen worm stocks. Plate #5, well H7, was

15   thawed and transferred to nematode growth medium (NGM) plates seeded with *E. coli*. Single animals were cloned on fresh NGM plates and the progeny were collected, lysed, and assayed for the presence of a DNA deletion by PCR using primer pairs identical to those described in Fig. 8. DNA from well H7 was included as a positive control. DNA from two worms, labelled 11 and 31, are

20   positive for the 1.3 kb deletion product (GIBCO-BRL, 1 kb ladder DNA molecular weight standards). The strain derived from progeny of the animal labelled 11 bore the deletion named *sel-12(nr2011)*.

Fig. 7 shows a schematic representation of the *sel-12* genomic region of wild-type and the nr2011-mutants. Boxes represent exons, lines between

25   boxes represent introns. The approximate locations of the PCR primers used in the screens are indicated by short horizontal bars and primer names. The location of the nr2011 deletion is indicated by the gap.

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Fig. 8 shows the location of the nr2011 deletion breakpoints. Relevant sequences from wild-type *sel-12* and *sel-12(nr2011)* genomic DNAs are shown. Arrows point to possible deletion breakpoints. The nr2011 deletion was formed either by breakage of wild-type sel-12 sequences after bp 2532 and bp 3958 or after bp 2534 and bp 3959.

Fig. 9 shows a schematic representation of the genomic region of predicted wild-type *hop-1* and *hop-1(nr2003)* deletion genomic regions. Boxes represent exons and lines represent introns. The approximate locations of the PCR primers used in the initial screening are indicated by short horizontal bars and primer names. Location of the nr2003 deletion is indicated by the gap.

Fig. 10 shows the location of the nr2003 deletion breakpoints. Relevant sequences from wild-type *hop-1* and *hop-1(nr2003)* genomic regions are shown. Arrows point to possible deletion breakpoints. The nr2003 deletion was formed by cleavage of wild-type *hop-1* sequences either after bp 2306 and 3502 or after bp 2307 and 3503.

Fig. 11 shows a diagram of phenotypes from *hop-1/+; sel-12/+* progeny.

Fig. 12 shows a schematic of a screen of the invention.

Fig. 13 shows the relative functions of mutations which may be identified using the nematodes and methods of the invention.

Fig. 14 is a schematic showing the potential interactions between gene products and genes which may be detected using the methods of the invention.

Description of the Preferred Embodiments

I. Creation of nematodes for the study of Alzheimer's Disease

To explore the functions of the *sel-12* and *hop-1* presenilins, we used

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a reverse genetic strategy to generate *sel-12* and *hop-1* deletion mutations. Our analysis of *hop-1;sel-12* double mutants provides genetic evidence that *sel-12* and *hop-1* function redundantly to promote Notch-pathway signaling and reveals a requirement for maternal expression of *hop-1* and *sel-12*. In addition,

5   we describe results of genetic screens to isolate suppressors of combinations of *sel-12*, *hop-1* and *spe-4* mutations and screens for mutations which phenocopy combination of *sel-12*, *hop-1* and *spe-4*. The nematodes used in these screens may also be used to screen for compounds which prevent, ameliorate, or exacerbate AD.

10   Results

A hop-1 deletion mutant in a wild-type genetic background has no striking phenotype. To demonstrate that *hop-1* is expressed, we isolated *hop-1* .cDNAs and performed reverse transcriptase-PCR and sequence analyses.

A deletion in the *hop-1* locus was then generated using a reverse.

15   genetic approach described below. Deletion libraries representing 460,000 mutagenized genomes were screened by PCR with primers flanking the *hop-1* coding region (see Example 1). Animals containing the deletion *nr2003* were recovered from a library that had been mutagenized with UV-trimethylpsoralen. Sequence analysis revealed a 1196 bp deletion beginning

20   434 bp upstream of the *hop-1* translation start site and ending in the third intron (Fig. 1A). *hop-1(nr2003)* homozygotes were viable and had no apparent morphological or behaviorial defects. However, at 15°C, *hop-1(nr2003)* hermaphrodite brood size was reduced by 44% relative to wild-type (147±288 vs. 262±130). Unlike mutations in *sel-12* (Levitan et al., Nature 377:351-354,

25   1995), *hop-1(nr2003)* did not noticably suppress a *lin-12* gain-of-function allele *n950sd*.

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   hop-1 and sel-12 are redundant for maternal and zygotic functions in
embryogenesis and germline proliferation. To examine whether *hop-1* and *sel-*
12 have redundant functions, we generated a *hop-1;sel-12* double mutant.
First, we isolated a *sel-12* deletion mutant (*nr2001*) using the same reverse

5   genetic strategy that was used to generate *hop-1(nr2003;* see below). Deletion
libraries representing 1,100,000 genomes were screened with *sel-12*-specific
primers (see *Example 1*). Animals containing the deletion *nr2011* were
recovered from a library mutagenized with ENU. Sequence analysis revealed a
1426 bp deletion starting in the second intron and ending in the sixth intron

10 (Fig. 1B); splicing of the second exon to the seventh exon, if it occurred, would
cause a frameshift. Thus, *sel-12(nr2011)* is predicted to encode a severely
truncated protein containing the N-terminal 82 amino acids of Sel-12 followed
by 23 novel amino acids. Like previously described *sel-12* mutations (Levitan
et al., Nature 377:351-354, 1995), *sel-12(nr2011)* caused a highly-penetrant

15 egg-laying defective (Egl phenotype and suppressed *lin-12(n950sd)*.

     hop-1;sel-12 double mutant animals displayed new phenotypes not
seen in either single mutant. Furthermore, the phenotype of double mutant
animals depended on maternal genotype (Table 1). Strains of three different
genotypes (designated A, B, and C) that segregate *hop-1;sel-12* progeny were

20 generated. Strain A, of the geneotype *hop-1/+; sel-12*, segregates *hop-1;sel-12*
double homozygotes that are sterile. In contrast, strain B, of the genotype *hop-*
1;sel-12/+, segregates *hop-1;sel-12* homozygotes that have a maternal-effect
embryonic lethal (Mel) phenotype and are Egl (the "Bag of Dead Eggs"
phenotype). Strain C, of genotype *hop-1/+; sel-12/+*, also segregates *hop-1;sel-*

25 *12* homozygotes that are both Mel and Egl (Table 1). We interpret these results
as follows: first, a complete absence of maternal and zygotic *hop-1* and *sel-12*
function results in embryonic lethality; second, in the absence of *sel-12*

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function, maternal expression of *hop-1* is sufficient for normal embryogenesis but not for normal germline development, resulting in sterility; and third, in the absence of *hop-1* function, maternal expression of *sel-12* is sufficient for normal embryogenesis and germline development.

5   *The phenotypes displayed by hop-1;sel-12 double mutants can be attributed to defects in GLP-1/LIN-12 signaling.* The sterile and Mel phenotypes displayed by *hop-1;sel-12* progeny are reminiscent of defects seen in *glp-1* loss-of-function (lf) mutants. Normally, a cell-cell interaction between the distal tip cell and germ cells, mediated by the GLP-1 receptor, induces germ

10   cells to proliferate (Austin et al., Cell 51:589-599, 1987). Strong *glp-1(lf)* mutants produce a reduced number of sperm, no oocytes, and hence are sterile due to the failure of their germ cells to respond this proliferative signal (Austin et al., Cell 51:589-599, 1987). Similarly, the gonads of *hop-1;sel-12* progeny that segregate from strain A contain 50-100 sperm but neither oocytes nor

15   undifferentiated germ cells (Table 1, Figs. 2A-2D).

Weak *glp-1(lf)* mutations have revealed additional roles for maternally-contributed *glp-1* in early embryonic development (Austin et al., Cell 51:589-599, 1987; Priess et al., Cell 51:601-611, 1987; Mello et al., Cell 77:95-106, 1994). At the 4-cell embryo stage, *glp-1* is required for proper fate-

20   specification of the blastomere ABp (Mello et al., 77:95-106, 1994). Similarly, we found that inviable *hop-1;sel-12* mutant embryos (derived from strain B) lack intestinal valve cells (Fig. 3A-C).

A second requirement for *glp-1* occurs at the 12-cell embryo stage (Priess et al., Cell 51:601-611, 1987; Mello et al., Cell 77:95-106, 1994). At

25   this stage, descendants of ABa are induced to produce pharyngeal tissue as a result of a *glp-1*-dependent cell-cell interaction. In *glp-1* mutant embryos, this interaction fails and the anterior lobe of the pharynx is not formed. However,

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the posterior lobe of the pharynx is present, because the cells that comprise it

are descended from a different blastomere, MS, that does not require a *glp-1*-

mediated interaction to produce pharyngeal cells (Priess et al., Cell 51:601-611,

1987). Like *glp-1* mutant embryos, inviable *hop-1;sel-12* mutant embryos lack

5   anterior pharynx but form posterior pharynx. *hop-1;sel-12* embryos were

stained with an antibody specific for pharyngeal myosin (see *Example 1*). The

amount of pharyngeal tissue seen in these embryos was reduced relative to that

seen in wild-type embryos and was comparable to that seen in *glp-1* mutant

embryos (Fig. 3D-F). Furthermore, as with *glp-1* mutant embryos (Priess et al.,

10   Cell 51:601-611, 1987), no pharyngeal tissue was observed in *hop1; sel-12*

embryos in which the posterior pharynx had been eliminated by killing

descendants of the MS blastomere. Taken together with the results showing the

absence of intestinal valve cells, these data suggest that cell-fate defects similar

to those observed in *glp-1* mutant embryos occur in inviable *hop-1;sel-12*

15   mutant embryos.

It was useful to demonstrate that the new phenotypes uncovered in

hop-1;sel-12 mutants were due to loss of *hop-1* activity. We used RNA-

mediated interference (RNAi) (Fire et al., Nature 391:806-811, 1998) to show

that disruption of *hop-1* function in *sel-12* mutant animals can lead to defects

20   that are similar to those observed in *hop-1(nr2003); sel-12* double mutant

animals. Antisense *hop-1* RNA was injected into both wild-type and *sel-12*

mutant hermaphrodites (see *Example 1*). Progeny from injected *sel-12* mutant

animals included both sterile animals with a germline proliferation (Glp) defect

and dead embryos, whereas progeny from injected wild-type animals had no

25   apparent defects (brood size was not scored). These results argue that the

defects observed in *hop-1(nr2003); sel-12* mutant animals are dependent on the

nr2003 deletion.

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In addition to Glp-1-like defects, some *hop-1;sel-12* mutants display Lin-12-like defects. *lin-12(lf)* mutants exhibit numerous cell-fate changes (Greenwald et al., Cell 34:435-444, 1983). One well-characterized cell-fate change occurs in the hermaphrodite somatic gonad. During normal larval

5   development, *lin-12*-mediated signaling between the somatic gonad primordium cells Z1.ppp and Z4.aaa ensures that they develop into two distinct comatic gonadal cell types, an anchor cell (AC) and a ventral uterine precursor cell (VU). However, in *lin-12(lf)* mutants, both Z1.ppp and Z4.aaa develop into ACs (Greenwald et al., Cell 34:435-444, 1983). We found that *hop-1;sel-*

10   *12* mutant animals segregating from strain A, but not from strain B, also have two ACs (Table 1, Figs. 2A-2D). Furthermore, *hop-1;sel-12* mutant animals segregating from strain A have a highly-penetrant protuding vulva phenotype that closely resembles that seen in *lin-12(lf)* mutants ((Greenwald et al., Cell 34:435-444, 1983) and Figs. 2A-2D).

15   Since *hop-1;sel-12* mutant animals from strain A display both *Glp-1*-like and Lin-12-like defects, we reasoned that they might also exhibit phenotypes similar to those of *lin-12; glp-1* double mutant animals. *lin-12; glp-1* double mutants display more severe defects than either single mutant, due to partial redundancy of *lin-12* and *glp-1* functions (Lambie et al., Development

20   112:231-240, 1991). The *lin-12; glp-1* double mutant phenotype, termed lag (*lin-12* and *glp-1*), is a zygotic larval lethal with characteristic cell fate defects. We did not detect any Lag-like animals among 485 progeny segregating from *hop-1/+; sel-12* hermaphrodites.

         Genetic suppressors of hop-1;sel-12 are much more difficult to

25   *isolate than are suppressors of sel-12 alone.* To identify genes that interact with *C. elegans* presenilin genes, we carried out three screens to suppress phenotypes caused by *sel-12* and *hop-1* mutations. The first screen was

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designed to isolate suppressors of the Egl phenotype of *sel-12* mutant

hermaphrodites (see *Example 1*). From 35,000 genomes screened, 56

independent suppressed lines were obtained, a frequency of 1 in 625 genomes.

       The second screen was designed to isolate suppressors of the sterile

5   phenotype of *hop-1;sel-12* double mutants derived from strain A. This screen

was complicated by the fact that the double mutant could not be maintained in a

homozygous state. To circumvent this difficulty, we took advantage of

mutations that confer resistance to the anthelminthic agent ivermectin, using

this to select for the one-quarter *hop-1;sel-12* doubly homozygous progeny that

10 segregate from a *hop-1/+; sel-12/sel-12* parent (see *Example 1*). From 77,000

genomes screened, no suppressors were identified.

       The third screen was designed to isolate suppressors of the Egl

phenotype of *hop-1/+; sel-12/sel-12* hermaphrodites. This screen offers several

advantages compared to the screen for suppressors of the *sel-12* Egl phenotype

15 in a *hop-1(+)* background. First, any suppressor mutations obtained can rapidly

be examined for their ability to suppress the *hop-1;sel-12* sterile phenotype by

examining *hop-1;sel-12* self-progeny of suppressed hermaphrodites. Both

strong suppression of the sterile phenotype, resulting in fertility, and weak

suppression, resulting in an increase in the number of germ cells or the

20 presence of oocytes, could be detected. Second, the Egl defect exhibited by

hop-1(nr2003)/+; sel-12(nr2011)/sel-12(nr2011) hermaphrodites is stronger

than that of *sel-12(nr2011)* hermaphrodites: 100% of

hop-1(nr2003)/+;sel-12(nr2011)/ sel-12(nr2011) hermaphrodites lay no eggs,

whereas 97% of *sel-12(nr2011)* hermaphrodites lay no eggs and the remaining

25 3% lay a small number of eggs, typically fewer than five. Therefore, we

anticipated that suppressors of the Egl phenotype of *hop-1/+; sel-12/sel-12*

animals might be more difficult to obtain than suppressors of the Egl phenotype

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of *sel-12* animals. Because suppressors of *sel-12* animals were frequent, a more stringent screen for suppression using the *hop-1/+;sel-12/sel-12* strain would be useful for choosing which suppressors to characterize further. From 30,000 genomes screened using the *hop-1/+;sel-12/sel-12* strain, nine

5   independent suppressed lines were obtained, a frequency of 1 in 3333 genomes. This frequency is indeed lower than that obtained using the *sel-12* strain. We have not detected suppression of the sterility defect of *hop-1;sel-12* animals in any of these lines.

Summary of Experimental Results

10   The finding that *hop-1; sel-12* mutant animals display *Glp-1* and *Lin-12* defects not observed in either single mutant indicates that *hop-1* and *sel-12* function redundantly to promote Notch-pathway signaling.

          Suppressors of the *sel-12* single mutant are found much more readily than are suppressors of the *hop-1; sel-12* double mutant. There are several

15   possible explanations for the marked difference between the ease with which we found suppressors of the *sel-12* Egl defect and our inability to find suppressors of the *hop-1;sel-12* sterility. First, *sel-12* Egl suppressors might promote egg-laying competence by activating *hop-1* function. Second, they might potentiate *glp-1/lin-12* signaling supported by wild-type levels of *hop-1*

20   activity, but unable to bypass the more severe reduction in *glp-1/lin-12* signaling associated with a complete lack of presenilin function. Third, *sel-12;egl-1* suppressors might affect egg laying through a mechanism independent of *glp-1/lin-12* signaling. Finally, suppressors of the *sel-12* Egl defect might be expressed in the soma but not in the germ line.

25   Our experiments conducted in *C. elegans* have revealed that presenilins facilitate Notch receptor function, although the precise role of

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presenilins in promoting Notch signaling has not yet been identified.

The relationship between the proposed role of presenilins in AD
pathogenesis—affecting the proteolytic processing of APP and their role in
promoting Notch receptor function—is not yet clear, but may be addressed using
5   our model system. Intriguingly, it has recently been demonstrated that the
Drosophila Notch receptor undergoes proteolytic processing by Kuzbanian, an
ADAM-family protease, *en route* to the cell surface, and that this cleavage
event is required for Notch to function appropriately. Thus, two proteins with
which presenilins are proposed to interact with (directly or indirectly), APP and
10   the Notch receptor, are both undergo proteolytic processing while trafficking to
the plasma membrane. Thus, presenilins might regulate Notch receptor
processing in a manner analogous to their regulation of APP processing. An
understanding of how presenilins affect Notch receptor activity is thus be
relevant to understanding how presenilin dysfunction causes AD.

15   Isolation of suppressors of hop-1, sel-12, and spe-4 and isolation of molecules
acting in the AD pathway

Our results suggest specific screens for the identification of targets
for the treatment of AD (see Figs. 12 and 13, and the Examples below). If, for
example, the PS(FAD) mutations are loss-of-function, negative regulators of a
20   presenilin pathway are potential drug targets. Thus, suppressors of presenilin
deletion knockouts will provide the following: 1) mutations that suppress
presenilin deletion knockouts and bypass the need for presenilins should
identify genes that function either downstream of presenilins or in a parallel
pathway; 2) recessive mutations that confer suppression are likely to be loss-
25   of-function mutations and should identify genes that are negative regulators of
a presenilin pathway or a parallel pathway; and 3) chemical suppressors are

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likely to inhibit negative regulators of a presenilin pathway or a parallel

pathway.

If, on the other hand, the PS(FAD) mutations are gain-of-function,

positive regulators of a presenilin pathway are potential drug targets.

5   Suppressors of presenilin deletion knockouts can provide dominant mutations

that suppress presenilin deletion knockouts such dominant mutations may be

gain-of-function and thus identify positive regulators of a presenilin pathway,

or a parallel pathway.

All deletion suppressor screens conducted to date have been F2

10   screens which have enabled isolation of both recessive and dominant

mutations. (F3 screens are also possible.) Preliminary evidence suggests that

some *hop-1/+;sel-12* suppressors may act semi-dominantly or dominantly.

If any suppressors have phenotypes alone, one may suppress that

phenotype, thus providing targets in the opposite direction of the pathway.

15   Suppressors of presenilin gain-of-function phenotypes will, in turn,

yield recessive mutations that confer suppression and are loss-of-function. Such

mutations should thus identify positive regulators of presenilins and chemicals

that confer suppression would likely inhibit positive regulators of presenilins.

Genetic Screens for Mutants That Phenocopy Presenilin Deletions

20   Phenocopying presenilin loss-of-function phenotypes will provide

the following information: recessive mutations that phenocopy presenilin loss-

of-function phenotypes are likely to be loss-of-function themselves, and thus

identify positive regulators of presenilins (or are presenilin mutants) and

chemicals that phenocopy presenilin loss-of-function phenotypes likely inhibit

25   presenilins or positive regulators of presenilins. A pilot genetic screen of 4800

genomes looking for "bags of dead eggs" phenotype displayed by some *hop-*

-19-

1;sel-12 hermaphrodites yielded eight potential mutants.

To find further genes which positively regulate one or more presenilin genes one may mutagenize worms and look for animals that phenocopy the presenilin mutants (e.g. have the phenotype of a single, double, or triple mutant, as described herein).

The first method is to mutagenize wild-type worms and look for "bags of dead eggs" to identify genes that positively regulate *hop-1* and *sel-12*. If such a gene also regulates *spe-4* they would be missed due to a Spe sterile phenotype. One may validate the above screen by analyzing dead embryos for Glp-like defects and testing for suppression of *lin12(gf)*.

A second method to achieve the above goal is to identify mutagenized wild-type worms and look for bags of oocytes that become bags of dead eggs when mated to wild-type males, phenocoping the triple a presenilin mutant. Such a screen could identify genes that positively regulate *hop-1, sel-12* and *spe-4*, a more restrictive screen, then the above screen. We predict that mutants will retain oocytes in the uterus, necessitating looking for striping of oocytes in the gonad on plates lacking oocytes on the plate.

A third method for identifying genes which phenocopy a presenilin deletion is to mutagenize *hop-1* worms and look for "bags of dead eggs." This is less stringent screen then the screen for wild-type worms with "bags of dead eggs" (above) and, in addition, identify positive regulators of *sel-12* only and new *sel-12* mutations. This adaptation would miss genes that regulate *sel-12* and *spe4*. However, such a gene would fail to interact with *hop-1* and so it might be picked up in the screen for "bags of dead eggs" (above).

A fourth approach is to mutagenize *sel-12* worms and look for Glp steriles. This screen is less stringent, but could isolate everything in the first two above screens, as well as positive regulators of *hop-1* only and new
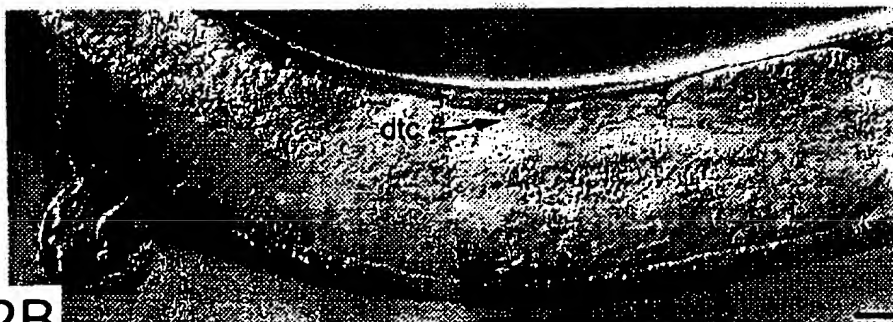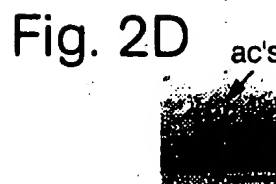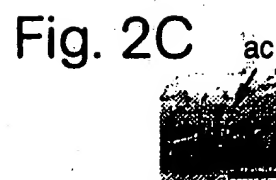
Fig. 2A

Fig. 2B

Fig. 2C

Fig. 2D

N2

glp-1

hop-1;sel-12

Fig. 3A

Fig. 3B

Fig. 3C

Fig. 3D

Fig. 3E

Fig. 3F

R O3E1-Akt

F1 F2 F3 F4 — Wild type
R4 R3   R2   R1

F1 F2 F3 F4 — mutant
R4 R3   R2   R1

1   2   3   4   5   6 kb

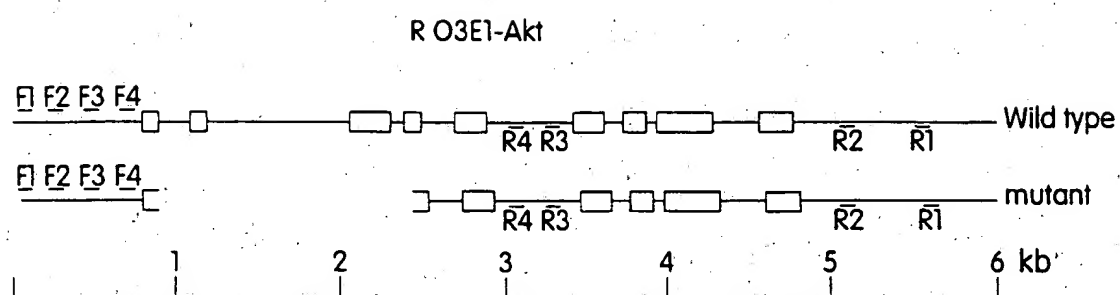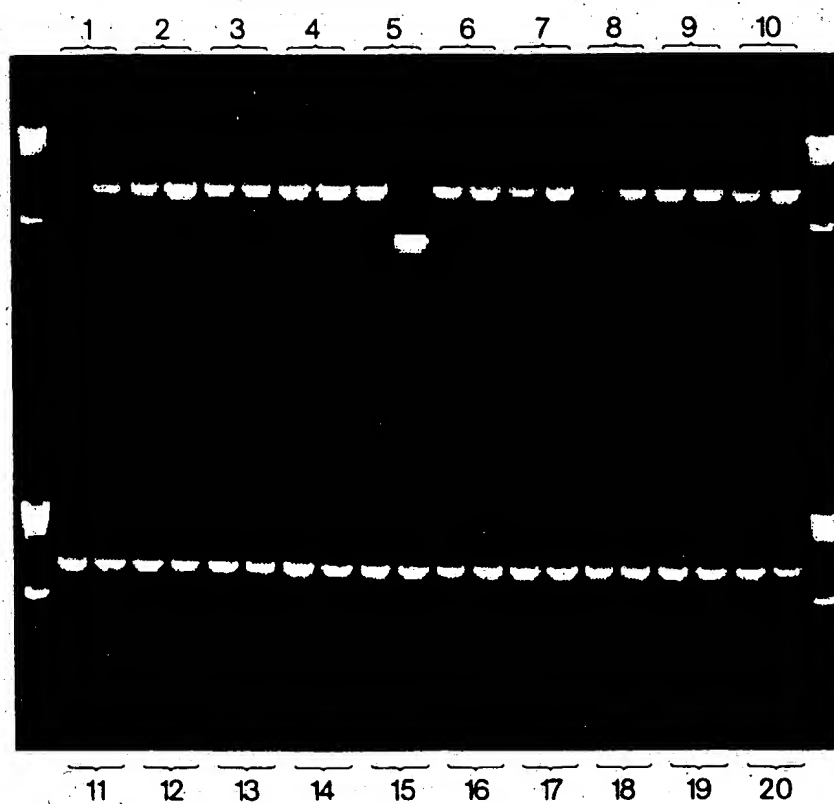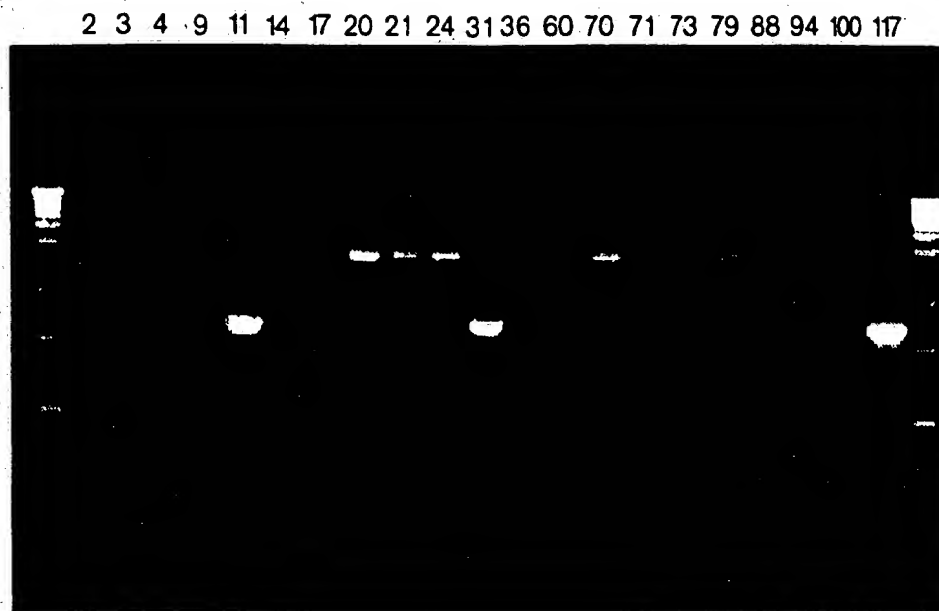# Fig. 4

Fig. 5

Fig. 6

sel-12
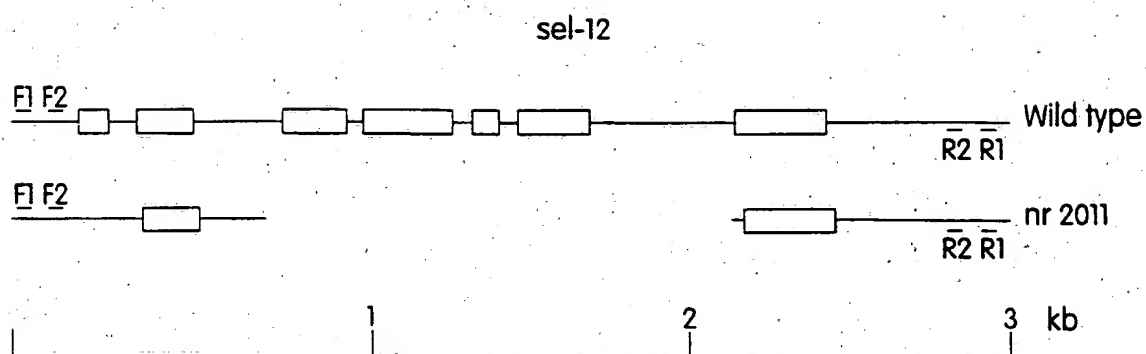

Fig. 7

sel-12(+) bp 2525-2545 TCTATCAA T ATTTCTTTGGAA

sel-12(+) bp 3955-3975 CATA T TTTTTTTAACAATAAT

sel-12(nr2011) bp 2525-2545 TCTATCAATTTTTTTTTAACAA

Fig. 8

hop-1

Fig. 9

hop-1(+) bp 2300-2500 AAACCAC A CACTTTCTAGACA
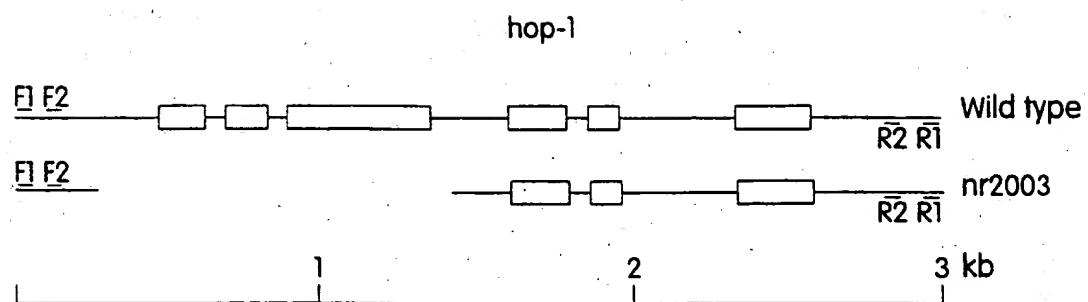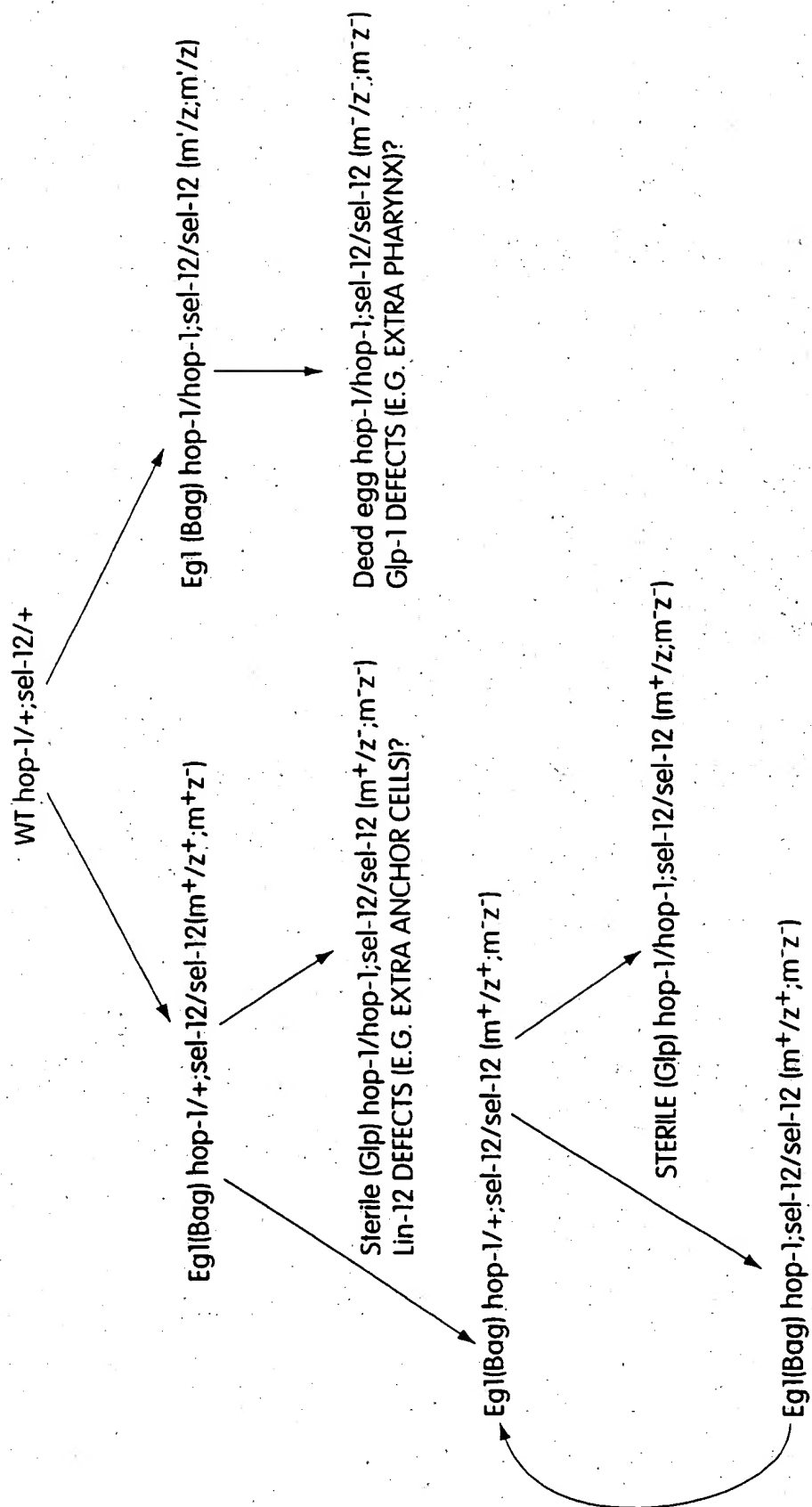
hop-1(+) bp 3490-3510 GCTACACAATAAA A GCCCAAA

hop-1(nr2003) bp 2300-2500 AAACCACAGCCCAAAATATAA

Fig. 10

Fig. 11

N2

↓

Place individual F1's in wells

Grow with food
to last four days
only

Screen wells for egg-containing adults

Validation

1. Verify sibs throw Bags of Dead Eggs.
2. Test dead embryos for Notch defects
3. Test for suppression of lin-12 (D)

Fig. 12

FAD presenilin mutations are:

	Loss of function	Gain of funcction
Relevent Suppressor Mutations	Loss-of-function (LOF)	Gain-of-function (GOF)
Relevent Revertants of LOF Suppressor	GOF	LOF
Relevent Revertants of GOF Suppressor	LOF	GOF
Relevent Phenocopy Mutations	GOF	LOF

Fig. 13

Potential Targets if FAD mutations are Loss-of-Function (LOF)

LOF Suppressor of PS

GOF PS Mimic

Presenilins

lin-12/glp-1

GOF Suppressor of PS

LOF PS Mimic

Potential Targets if FAD mutations are Gain-of-Function (GOF)

(In all cases, implied interactions may be indirect)

Fig. 14

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 5/00, 15/00; A61K 49/00; C12Q 1/70
US CL :800/8, 9, 12, 13; 424/9.2; 435/5, 172.3; 536/23.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 800/8, 9, 12, 13; 424/9.2; 435/5, 172.3; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CHEMICAL ABSTRACTS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	L'HERNAULT, S.W. et al. Mutation of a Putative Sperm Membrane Protein in Caenorhabditis elegans Prevents Sperm Differentiation but Not Its Associated Meiotic Divisions. Journal of Cell Biology, October 1992, Vol. 119, No. 1, 55-68, especially pages 56 and 58-60.	1-12 and 16-18
Y	LEVITAN, D. et al. Assessment of Normal and Mutant Human Presenilin Function in Caenorhabditis elegans. Proceeding National Academy of Sciences (USA). December 1996, Vol. 93, pages 14940-14944, especially pages 14942-14944.	1-15

[X] Further documents are listed in the continuation of Box C.   [ ]   See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"B"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 AUGUST 1998	1 9 AUG 1998

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Form PCT/ISA/210 (second sheet)(July 1992)*

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LEVITAN, D. et al. Facilitation of lin-12-Mediated Signalling by sel-12, a Caenorhabditis elegans S182 Alzheimer's Disease Gene. Nature. 28 September 1995, Vol. 377, pages 351-354, especially pages 352-354.	1-12 and 16-18
Y	NAITO, M et al. Identification of a Homeobox-Containing Gene Located Between lin-45 and unc-24 on Chromosome IV in the Nematode Caenorhabditis elegans. Nucleic Acids Research. 1992, Vol. 20, No. 12, 2967-2969, especially 2967.	16-18

-20-

mutations in *hop-1, glp-1, lag-1,* and *lag-2.*

Screens for Compounds That Phenocopy Presenline Deletions

         To determine if a compound will affect one, two, or all three presenilins and to determine at what stage a compound should be added in
5   order to see Glp sterile, Spe sterile, Egl and Mel phenotypes one may do the following: 1) add compound to wild-type worms (L1s and L3s/L4s or a mixed population) and look for Glp steriles and bags of dead eggs or Glp/Spe steriles and Spe steriles (this assumes a chemical affects HOP-1 <u>and</u> SEL-12 (directly or indirectly), but may or may not affect SPE-4); 2) add the compound to
10   wells of L3/L4 *hop-1* mutant or wild-type worms and look for "bags of dead eggs" in the *hop-1* wells, but not the corresponding wild-type wells (this assumes a compound affects SEL-12, but not HOP-1 or SPE-4); 3) substitute human PS1 for *sel-12* or for *hop-1/+;sel-12,* then do screen of 1), above (if presenilins are targets, then humanizing the worms in this way improves the
15   screen); or 4) add compounds to wells of L3/L4 *sup (hop-1/+;sel-12 Egl sup)* mutant or wild-type worms and look for "bags of dead eggs" in the wild-type wells, but not in the *sup* wells.

II. Reverse Genetics Strategy

         We have developed a technique for the rapid isolation of new strains
20   of nematodes that have a deletion in essentially any gene or other region of choice. This technique provides a rapid method for reverse genetics in this powerful model organism.

         In our reverse genetics strategy, large numbers of mutagenized worms are distributed into an ordered array of separate cultures, which are
25   grown to generate numerous progeny. Genomic DNA is prepared from an aliquot of each culture of worms, while the remaining worms are stored away

-21-

as viable sibling populations. PCR is then used to screen for deletions within a targeted gene, using primers. Primers may readily be designed, for example, from publicly available DNA sequence generated by the *C. elegans* Genome Sequencing Consortium. Amplicons from a template bearing deleted DNA

5   within the targeted region will be shorter and thus identify a specific address within the ordered array of cultures. "Knockout" animals bearing such deletions are then isolated from that specific culture, and studied with the full range of biological approaches to elucidate the function of the targeted gene.

We have successfully identified mutations in every region of choice

10   which has been targeted to date. Our reverse genetics technology provides new tools for functional genomics approaches Such approaches may be pursued together with standard genetic, molecular, and pharmaceutical methods in order to illuminate the function and pathway of conserved genes that are involved in human diseases and other diseases of interest.

15   Protocol for General Mutagenesis:

1. The first step is to mutagenize the nematodes. Deletions have been identified following mutagenesis with EMS, ENU, diepoxyoctane and UV/trimethylpsorlalen (see the examples, below). We believe a variety of other mutagens known to mutagenize the nematode genome may also be

20   employed at this step. We estimate the efficiency of mutagenesis by determining the number of ivermectin-resistant mutants in the F2 generation.

2. Following mutagenesis, cultures are started by seeding twenty F1 worms into each well of a microtiter plate. Following growth of the cultures, each well contains approximately 2000 F2 larvae. Our standard-sized library

25   contains forty-eight 96-well microtiter plates, representing 192,000 mutagenized genomes. Thus far we have screened for deletions using seven

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such libraries, three mutagenized with UV/TMP, two with EMS and one each with ENU, and diepoxyoctane.

3. For each well, one-third of the worms are separated into two equal portions and digested with worm lysis buffer to produce two sets of DNA extracts. The remaining two-thirds of the worms are frozen.

4. For the primary screen, the two sets of DNA extracts are screened in parallel. We screen in duplicate to avoid cases where a deletion has occurred in a single F2 worm. For each set of DNA extracts, an aliquot of the extract from each well of a 96-well plate are mixed, generating a pool representing 4,000 mutagenized genomes. Our PCR protocol is based on a modification of protocols used for Tc1 mutagenesis (See e.g., in Caenorhabditis elegans: Modern Biological Analysis of an Organism, Methods in Cell Biology, Volume 48, pp. 59-80 incorporated herein by reference) and uses a nested set of primers generally placed 3-5 kb apart, AmpliTaq Gold (Perkin-Elmer), and approximately 35 cycles of amplification are generally used. For most of our screening, we adjust the extension times (usually 2-3 min) such that a faint wild-type band is generated. We have also used extension time such that no wild-type band is generated. Once a potential deletion band has been identified by its altered size, the pool is tested four more times to determine whether a deletion band is actually present in the pool. After a deletion band has been replicated, a secondary screen is performed on pools of row and columns from the microtiter plate to identify the well containing the deletion and hence the well of frozen animals containing nematodes carrying the deletion.

5. Frozen worms from the well with the deletion are thawed (about 500 animals are usually recovered), cloned, and screened by PCR for animals carrying the deletion.

6. The worm having the desired deletion may then be characterized

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genetically and biochemically and used for therapeutic drug screening and

research protocols.

Yield

This method has been used to isolate worms carrying deletion

5   mutations in multiple genes. The average frequency of successful isolation is

about one deletion from 400,000 genomes screened. With primers set 3-3.5 kb

apart, most deletions detected are between 500 and 1500 bp size. Larger

deletions have been detected with primers set 5 kb apart.

Frequency

10   Among the thawed worms, one would expect 3 in 80 animals to

carry a viable deletion mutation (1/20 x 3/4) and 1 in 30 animals to carry a

lethal deletion mutation (1/20 x 2/3). However, in practice we have found that

it is advisable to screen a minimum of 250 animals since the frequency of

animals carrying deletions can vary.

15   EXAMPLES

The following examples are meant to illustrate, not limit the

invention.

Example 1: Materials and Methods

General methods and strains. Nematode strains were cultured using

20   standard techniques (Brenner, Genetics 77:71-94 1974). except that strains

used to generate the deletion libraries were cultured in liquid, as described

below. All strains were grown at 20°C unless noted otherwise. *C. elegans*

variety Bristol strain N2 was the parent of all strains used in these studies

(Brenner, Genetics 77:71-94 1974). Alleles used were: *LGI: unc-73(e936),*

25   *hop-1(nr2003), avr-14(nr391), dpy-5(e61); LgIII: lin-12(n950sd), glp-*

1(q231ts); LGV: avr-15(nr395); LGX: egl-17(e1313), pha-2(ad472), sel-

-24-

12(nr2011), unc-1(e538), dpy-3(e27)

     Generation of the deletion libraries. For mutagenesis, late fourth-
larval-stage (L4) N2 hermaphrodites were either treated with one of the
following three chemical mutagens for 4 hours: ethyl methane sulfonate (EMS)

5   at 0.25%, ethylnitrosourea (ENU) at 0.4 mM or diepoxyoctane (DEO) at 1 mM
(Anderson, in Caenorhabditis elegans: Modern Biological Analysis of an
Organism, eds. Epstein and Shakes, Academic Press, Inc., San Diego, 48:31-
58, 1995; and refs within), or exposed to UV light (366nm) at 3.5 mW/cm^2 for
15 to 30 seconds after a 20 minute incubation in 30 μg/ml trimethylpsoralen

10   (Yandell et al., Proc. Natl. Acad. Sci. USA 91:1381-1385, 1994). F_1 eggs
derived from mutagenized hermaphrodites were collected for 4 hours, then
allowed to hatch for 16 hours. For each deletion library, hatched larvae were
distributed to 48 96-well polystyrene microtiter plates at about 20 larvae per
well in NGM medium (Wood, Cold Spring Harbor Laboratory Press,

15   Plainview, N.Y., 1988) with 1% (v/v) *E. coli* HB101 as food. Each library thus
represents approximately 2 x 10^5 mutagenized genomes. Worms were cultured
in wells for about five days until no food remained, at which point
approximately 100 F_2 progeny per F_1 animal had been produced. Half of the
worms in each well were removed and used to make genomic DNA. Of these,

20   half were transferred to wells of V-bottom 96-well microtiter plates and half
were pooled; for each of the original microtiter plates, worms from all wells
were combined into a single pool (plate pools). Worms in V-bottom wells were
digested with 10 μl worm lysis buffer (Williams et al., Genetics 131:609-624,
1992) for up to 16 hours. Plate pools were digested for 4 hours with 0.4 ml

25   plate pool lysis buffer (100 mM NaCl, 100 mM Tris-Cl (pH 8), 50 mM EDTA,
1% SDS, 1% β-mercaptoethanol and 100 μg/mL proteinase K). The worms
remaining in the original microtiter plates were frozen using standard methods

-25-

and served as viable stocks (Wood, Cold Spring Harbor Laboratory Press, Plainview, N.Y., 1988).

   Identification and recovery of hop-1 and sel-12 deletion mutants. Plate pools from multiple libraries were screened for deletions using nested

5   polymerase chain reaction (PCR) primers specific for genomic sequences flanking the *hop-1* or *sel-12* coding regions (see Fig. 5). Primer pairs were chosen such that they amplified wild-type products ranging from 2.5 to 3.5 kb in size. Primer sequences are shown in Tables 2 and 3. The extension time was determined empirically for each primer pair such that a faint wild-type

10   product was amplified reproducibly. Plate pools that gave rise to a deletion product were rescreened in quadruplicate to eliminate false positives. In cases where a pool was confirmed as positive, DNA from each well comprising that pool was screened individually by PCR. Once the specific well containing the deletion was identified, the corresponding well of worms was thawed and the

15   animals recovered. Survivors from each frozen well were transferred clonally to plates. Hermaphrodites were cultured until they laid eggs and then genotyped by single-worm PCR (Williams, et al., Genetics 131:609-624, 1992). Homozygous lines were established from the self-progeny of hermaphrodites containing the deletions. Sequence analysis of PCR products indicated that the

20   *hop-1 (nr2003)* deletion is 1196 bp and extends from bp #23,744 or 23,743 to 22549 or 22548, while the *sel-12(nr2011)* deletion is 1426 bp and extends from bp #5533 ro 5534 to 6958 or 6959 (Figs. 9-10). *hop-1*-related numbering refers to cosmid C18E3 (accession number AF000265) and *sel-12*-related numbering refers to cosmid F35H12 (accession number U41540). *hop-1(nr2003)* was

25   outcrossed ten times to an *unc-73(e936) dpy-5(e61)* strain and *sel-12(nr2001)* was outcrossed seven times to N2 before the genetic analyses described here were conducted.

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RNA-mediated interference (RNAi) experiments. The template used

to make RNA was an almost full-length *hop-1* cDNA (lacking seven bp at the

5' end) inserted into the vector pCR2.1 (Invitrogen). The insert and flanking T7

promoter were amplified by PCR using M13 forward (-20) and reverse primers.

5 Antisense *hop-1* RNA was transcribed from this PCR product *in vitro* using T7

polymerase and a Riboprobe kit (Promega), then microinjected into N2 or *sel-*

12(nr2011) hermaphrodite gonad arms at a concentration of 0.9 mg/ml using

the method of Mello et al., Embo J. 10:3959-3970 (1991). None of the N2

hermaphrodites (0/10) had progeny that displayed any morphological or

10 developmental abnormalities, whereas all of the *sel-12* hermaphrodites (8/8)

produced dead embryos and/or sterile progeny with a germline proliferation-

defective phenotype.

Laser killing of cells in embryos and antibody staining. Microscopy

and laser microsurgery were as described by Bowerman et al., Cell 68:1061-

15 1075 (1992), methanol/acetone fixation and freeze-crack permeabilization were

as described by Miller et al., in Caenorhabditis elegans: Modern Biological

Analysis of an Organism, eds. Epstein and Shakes, Academic Press, Inc.,

SanDiego, 48:365-394 (1995) and antibody staining and was as described by

Shi et al., Genes & Dev 12:943-955 (1998). To visualize intestinal valve cells

20 more easily, either the EMS or E blastomere was laser-killed to eliminate the

intestine, as both the valve cells and the intestine stain with the J126 antibody

(Mango et al., Development 120:2305-2315, 1994). For cell killing, early

embryos were dissected from gravid N2, *glp-1(q231ts)*, or *hop-1;sel-12*

parents. *glp-1(q231ts)* hermaphrodites had been shifted from 15°C (permissive

25 temperature) to 20°C (non-permissive temperature for the maternal-effect

lethal (Mel) phenotype (Austin et al., Cell 51:589-599, 1987)) 12-24h prior to

dissection. After cell killing embryos were incubated for 6h at 22°C, then

-27-

stained with the antibody. To visualize pharyngeal tissue, a monoclonal
antibody (9.2.1) that is specific for pharyngeal myosin (Epstein et al., in Muscle
Development, eds. Pearson and Epstein, Cold Spring Harbor Laboratory, Cold
Spring Harbor, N.Y., pp. 7-14 1982) was used. *glp-1(q231ts)* embryos were

5   taken from plates that had been incubated at 20°C for 24h; *hop-1;sel-12*
embryos were dissected from the Egl parents.

Suppression of the Egl phenotype of sel-12(nr2011 and hop-1(nr2003 /+; sel-12(nr2011) animals. *sel-12(nr2011)* and *hop-1(nr2003) avr-14(nr391)/unc-73(e936) dpy-5(e61); avr-15(nr395); sel-12(nr2011)*

10   hermaphrodites were mutagenized with EMS as described (Brenner, Genetics
77:71-94, 1974) and cultured individually on plates. Plates were screened 8 to
10 days later for the presence of F_3 eggs. Eggs were transferred to fresh plates
and the animals that hatched from them were tested for egg-laying competence.
Suppressor lines were established from single egg-laying competent F_3 animals.

15   *Suppression of the sterile phenotype of hop-1(nr2003); sel-12(nr2011)* animals. *hop-1(nr2003) avr-14(nr391)/unc-73(e936) dpy-5(ey1); avr-15(nr395) sel-12(nr2011)* hermaphrodites were mutagenized with EMS as
described (Brenner, Genetics 77:71-94, 1974). Mutagenized fertile non-Unc
non-Dpy animals were cultured individually in wells of 96-well microtiter

20   plates containing NGM medium with 1% (v/v) *E.coli* HB101 as food. When F_2
animals were first-or second-stage larvae, (typically 7-8 days later), the
contents of each well were transferred to an agar plate containing NGM
medium supplemented with 25 mM ivermectin. Plates were screened
periodically for fertile animals starting one week later and continuing for up to

25   four weeks. *avr-14(nr391)* and *avr-15(nr395)* single mutants are not resistant
to ivermectin, but *avr-14(nr391); avr-15(nr395)* double mutants are highly
resistant. Therefore, most of the animals that grew to adulthood on ivermectin-

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containing plates were of the genotype *hop-1(nr2003) avr-14(nr391); avr-15(nr395); sel-12(nr2011)* and were sterile. Animals containing a suppressor mutation were expected to be fertile and homozygous for the *hop-1* chromosome.

5   Example 2: Construction of *sel-12; hop-1* double mutant from nematodes having deletions in presenilin genes the two genes.

The power of the techniques of the invention are demonstrated by our subsequent ability to make a *sel-12; hop-1* doubly deleted worm having a surprising phenotype which makes it particularity useful for drug discovery.

10   The *hop-1* deletion constructed by our technique lacks a discernable phenotype in an otherwise wild-type background. Accordingly, absent the present technique, such a mutant would have been hard to obtain using standard genetic techniques.

Upon obtaining the *hop-1* deletion mutant and the *sel-12* deletion

15   mutant we constructed the *hop-1(nr2003), sel-12(nr2011)* doubly deleted mutant. While *sel-12* deletion mutants are Egl, we were surprised to find that the *sel-12; hop-1* doubly detected strain has a maternal lethal phenotype. This indicates these two genes have at least partially redundant functions. Thus, the double mutant provides a nematode useful for improved screens. These screens

20   may be used to identify suppressor mutations which, in turn, identify other genes in this Alzheimer's-related pathway. The screens may also be used to identify therapeutic compounds which ameliorate the effects caused by mutations in the presenilin genes.

We believe a *spe-4; hop-1* doubly deleted worm, a *spe-4; sel-12*

25   doubly deleted worms and the *sel-12; hop-1; spe-4* triply deleted worms (or parents capable of giving rise to eggs having these genotypes) will all be useful

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in the above described methods.

Fig. 11 shows the expected progeny, with phenotypes, for a *hop-1/+*; *sel-12/+* wild-type phenotype parent strain. Optimally, suppressors may be obtained by looking for revertants of the *hop-1/hop-1;sel-12/sel-12* (m-/Z-; m-

5   Z-) dead egg phenotype or the sterile phenotype of the genotypically identical (m+/Z-; m-; Z-) animals. Therapeutic compounds may be screened by providing the test compound at the appropriate stage (i.e., exposing the parent of the generation having the sterile or dead egg phenotype to the test compound), if necessary.

10   One skilled in the art of nematode genetics will recognize that dominant and negative linked markers, balancer chromosomes, and plating techniques may be employed to enhance the efficiencies of the methods, as needed or desirable.

Example 3: Genetic Screens for Alzheimer's Disease-Associated Genes

15   If the presenilin mutations which are causative for Alzheimer's are reduction-of-function mutations, then loss-of-function mutations which suppress presenilin reduction of function mutations in the worm will identify molecules whose inhibition could ameliorate the disease state in humans. Gain-of-function mutations which mimic presenilin reduction of function may

20   also identify such molecules (see Fig. 13).

Suppression of presenilin loss-of-function mutations

spe-4 causes a defect in spermatogenesis. *sel-12* causes an egg-laying defect. *hop-1* causes a mild reduction in brood size. The *hop-1; sel-12* double mutation results in different phenotypes depending on maternal

25   genotype. If the mother of the animal was either *hop-1/+; sel-12/+* or *hop-1; sel-12/+*, than the double mutant animal will be egg-laying defective and the

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eggs will die, a "Bag of Dead Eggs" phenotype. If the mother of the animal was *hop-1/+;sel-12*, then the double mutant animal will be Glp-Sterile. Additionally, a *hop-1/+;sel-12* animal has a stronger egg-laying defect than that caused by *sel-12* alone.

5   Specific screens are as follows.

We have conducted an F2 screen for suppressors of the Glp Sterile double mutant animals form the *hop-1/+;sel-12* mothers. This screen could have recovered both recessive, semi-dominant, or dominant suppressors. In 77,000 genomes screened, 0 suppressors were identified.

10   One may conduct an F3 screen for suppressors of the Glp Sterile double mutant animals from the *hop-1/+;sel-12* mothers. This screen could recover mutations which are materially-rescued, and therefore would not be identified in an F2 screen.

We have conducted an F2 screen for suppressors of the egg-laying

15   defect of *hop-1/+;sel-12* animals. This screen recovered recessive and semi-dominant mutations, and could have recovered dominant mutations as well. In 30,000 genomes screened, 9 suppressors were recovered.

One may conduct an F1 screen for suppressors of the egg-laying defect of *hop-1/+;sel-12* animals. This screen will recover semi-dominant and

20   dominant mutations, which could define either gain-of-function, haplo-insufficient or dominant-negative mutations.

One may conduct an F2 screen for suppressors of the "Bag of Dead Eggs" double mutant animals from the *hop-1;sel-12/+* and *hop-1/+;sel-12* mothers. This screen could recover recessive, semi-dominant, or dominant

25   suppressors. If suppressors are identified, an F1 screen will be done to identify only suppressors which act dominantly or simi-dominantly. If suppressors are rare, than an F3 screen may be done to identify mutations which are

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maternally-rescued.

Screens which mimic presenilin loss-of-function

        To identify targets involved in both *hop-1* and *sel-12* activity, we

have conducted a clonal F2 screen of wild-type animals to identify mutations

5   which mimic the "Bag of Dead Eggs" phenotype of the *hop-1;sel-12* animals

from *hop-1/+;sel-12/+* mothers. We have screened 4800 genomes and have 8

potential suppressors. To determine if these mutations are involved in

presenilin function, we are determining if the embryonic lethality is similar to

the presenilin embryonic lethality. One may also test whether the mutations

10   phenocopy presenilins in their interactions with *lin-12(gf)*.

        One may conduct a clonal F2 screen of *lin-12(gf)* animals to identify

mutations which mimic the "Bag of Dead Eggs" phenotype, and which

suppress *lin-12(gf)*.

        To identify targets involved in *spe-4, hop-1* and *sel-12* activity, one

15   may conduct a clonal F2 screen for spermatogenesis-defective animals, which

when mated into give a "Bag of Dead Eggs" phenotype.

        To identify targets involved only in *sel-12* activity, one may conduct

a clonal F2 screen of *hop-1* animals to identify mutations which mimic the

"Bag of Dead Eggs" phenotype.

20   To identify targets involved only in *hop-1* activity, one may conduct

a clonal F2 screen of *sel-12* animals to identify mutations which mimic the Glp

Sterile phenotype.

Example 4: Nematode Mutagenesis

25 1.   Mutagen Treatment.

        Mutagenesis is performed on synchronous populations of L4 *C.*

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elegans larvae. Treatment of L4 animals is designed to maximize the exposure of germ cell precursors to mutagen treatment. A synchronous population of L4 animals is generated by either (i) treating a population of nematodes containing egg-bearing hermaphrodites with alkaline hypochlorite and placing the eggs

5   (which survive the treatment) onto fresh Nematode Growth Medium plates seeded with *E. coli* for 52 hours at 20°C or (ii) placing L1 onto fresh NGM plates seeded with *E. coli* for 48 hours at 20°C.

Treatment with potentially mutagenic chemicals is performed by dissolving or diluting the pure mutagen to the selected concentration in

10   phosphate buffered saline and mixing the freshly, hatched L1 larvae (See e.g., Methods in Cell Biology, Volume 48, 1995 incorporated herein by reference (Eds. Epstein and Shakes, Academic Press, San Diego). The suspension of nematodes in mutagen is placed on a rocker table (to maintain mixing and aeration) for 3.5 to 4 hours at 20°C. The nematodes are then washed 3X with

15   PBS and distributed to fresh seeded NGM plates. Progeny to the treated animals carry the newly induced mutations.

UV/TMP Variation:

Mutagenesis with ultraviolet radiation following treatment with trimethylpsoralen (TMP) is performed by first mixing L4 larvae with 0.3 mM

20   TMP in PBS, 1% DMSO. After 15 minutes (in the dark) treated nematodes are transferred to an unseeded NGM plate in a small volume. After the liquid is absorbed (usually 5-10 minutes) the plates are irradiated with a calibrated UV lamp essentially as described by Yandall et al. (Proc. Natl. Acad. Sci., U.S.A., Vol. 91:1381-1385 (1994), incorporated herein by reference).

25   2. Monitoring mutagen treatment.

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To monitor mutagenesis, one measures (i) the lethality of the potential mutagen to treated animals, (ii) the frequency at which their (F1) progeny fail to hatch from eggs and (iii) the frequency of ivermectin resistance among the F2 progeny. Determination of that fraction of single gene detectable deletions may be done using standard statistical techniques.

(i) Lethality of the mutagen to treated animals is determined by observing the treated animals by dissecting microscope 24 hours after exposure to mutagen treatment is completed. Animals that are dead, have failed to grow, or have no fertilized eggs within their gonads are judged to have been excessively affected by the mutagenesis.

(ii) The fraction of F1 eggs that fail hatch is determined by washing the mutagen treated animals off of the NGM plates, leaving unhatched eggs adhered to the agar surface. Afer 24 hours the plates are washed again and the number of hatched larvae and unhatched eggs are determined by counting.

(iii) Ivermectin is an anthelminthic drug used to control parasitic nematodes that infect livestock, pets and humans. Exposure of eggs of wild type *C. elegans* to NGM plates containing concentrations of ivermectin > 2.5 microg/ml prevents the growth of *C. elegans* larvae. Selection of mutants that are resistant to the growth inhibiting effects of ivermectin provides a powerful screen for drug resistant mutants in *C. elegans*. Resistance to low levels of ivermectin (growth on 5-10 microg/ml of drug) is a phenotype conferred by single recessive mutations of more than 30 different genes. The frequency of low level ivermectin resistance thus acts as a convenient measure of the efficiency of mutagenesis leading to heritable genetic changes confined to single genes.

To measure the frequency of ivermectin resistance among populations of animals treated with a potential mutagen, we distribute 12 to 15

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progeny (F1) of the mutagen treated animals per well into 96 wells of a first

microtiter plate and, in a second plate, place 12 to 15 progeny of

unmutagenized as a control. The nematodes are distributed as small larvae,

suspended in liquid nematode growth medium seeded with *E. coli* as food. The

5   microtiter plates are incubated at 20°C until the larvae have grown to adults and

produced several hundred eggs and about 100 larvae (F2 animals) per well,

usually in about two days. (This number of progeny insures that all genomes

will be represented in the F2's before F1's are exposed to ivermectin. The

nematodes are not allowed to exhaust their food supply or to produce more than

10   200 larvae per well as the ivermectin resistance selection is less effective when

a larger number of worms are present.

After two days, one counts the number of F1 adults in 24 wells of

each plate. The mean, multiplied by 96, gives an estimate of the total number

of F1's in each plate. This value multiplied by two gives an estimate of the

15   total number of genomes tested for resistance. The contents of each well are

transferred to individual NGM plates seeded with E. coli and containing 7

ng/ml ivermectin. The plates are incubated at 20 to 23°C and examined weekly

for 4 weeks for the presence of viable, ivermectin resistant clones of animals.

Those plates scored as resistant typically have several thousand worms growing

20   on them including adults with eggs and these populations eventually

completely consume the bacteria/food. Plates without resistant animals are

generally devoid of viable animals; the original worms fail to grow and the

culture eventually dies out. The number of plates that contain a resistant clone

divided by the number of total number of genomes tested gives the ivermectin

25   resistance frequency.

(iv) We have developed a strategy to measure the fraction of single

gene mutations that are detectable deletions. This is important to optimize both

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mutagenesis and detection. The efficiency at which a mutagen treatment
induce heritable genetic changes conferred to single genes (measured by the
frequency of mutation to ivermectin resistance) multiplied by the fraction of
single gene changes that result from detectable deletions provides the desired
5   measure of the overall frequency at which the treatment generates detectable
deletions.

The current approach involves identifying alleles of known genes
and using PCR or Southern blotting to determine whether the alleles result from
detectable deletions. Ivermectin resistance again provides a convenient tool for
10   rapidly isolating alleles of known genes. Loss of function, including null,
alleles in two genes, *unc-33*, and *unc-44*, confer both ivermectin resistance and
a strong, highly characteristic, morphological and behavioral phenotype. We
can isolate 25 to 50 *unc-33* or *unc-44* alleles for each mutagen treatment and
determine with fraction of the alleles are conferred by detectable deletions.

15   3. Characteristics of an appropriate mutagen treatment.

The primary requirement of a mutagen treatment appropriate to use
in the invention is that the mutagen generate a useable frequency of modest
sized deletions (preferably between 0.5 and 3 kb). A useable frequency is
defined as at least one mutation in 1,000,000 gemones screened. The most
20   appropriate treatment will generate that highest frequency of detectable
deletions. Other desirable characteristics include: (i) low toxicity to treated
animals (i.e., less than 50%), and (ii) low level of killing of F1 eggs (i.e., less
than 50%).

4. Appropriate mutagen treatments.

25   Table 4 lists five mutagens, which have proven effective based on

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the mutagen monitoring assays provided herein (see above). Libraries made
with EMS, ENU, UV/TMP and DEO have all produced detectable deletions;
DEB has not yet been used to make a library but we predict it will yield
deletions.

5   Rejected Mutagens: Formaldehyde, chlorambucil, acrylamide, di-
epoxyoyclooctane, hexamethylphosphoramide, and methylene-bis-acrylamide
are specifically excluded from the methods of the invention. We have no
evidence that these compounds produce single gene mutations based on results
of the ivermectin resistance assay. We presume that these excluded mutagens
10   are either not mutagenic in *C. elegans* under the conditions of the invention
protocol, or that they produce larger, multigenic deletions or rearrangements
which are not detected by our assay. Chlorambucil and particularly
formaldehyde are also highly toxic to the treated worms. None of these
compounds have been used to produce mutagenized libraries.

15   <u>Example 5: Screen for deletions in Akt genes in *C. elegans*</u>
          The Akt gene encodes a serine-threonine protein kinase that is a
critical mediator of growth factor-induced neuronal survival (Science 275:661-
665, 1997). It has also been implicated to play a role in aspects of a number of
other human diseases. We sought to knock out a homologue of the Akt gene
20   located on cosmids R03E1 of *C. elegans* by screening for deletions using the
polymerase chain reactions (PCR).

<u>*Deletion screen in the Akt gene on R03E1*</u>:
          Two set of primers (see Table 5, primer list), each comprising of two
nested primer pairs, were chosen to screen mutagenized worm libraries by PCR
25   for deletions in the Akt gene on R03E1. A total of 352,000 mutagenized

genomes were screened in duplicate sets using the nested primers that were set approximately 3 kb apart. One potential deletion was identified from a UV-TMP mutagenized library. A second primer set was also used to screen the UV/TMP library. The second set of primers were placed approximately 5 kb

5   apart and used to screen the UV/TMP library. One potential deletion was identified from the same pool (pool 36) from which the other deletion was found. These two deletion-containing bands are approximately 0.8 and 2.7 kb in length. The location of the primers (see Table 5 and Fig. 4) and the sizes of the deletion-containing bands suggest that the two independent screens using

10   the two sets of primers identified the same deletion. Restriction mapping and sequencing may be performed to test this.

          To confirm that the deletion bands represent true deletions, pool 36 was tested by PCR again for the presence of the deletion bands. Four independent PCR reactions were performed from each of the duplicate pool 36

15   using identical primers as in the initial screens. All but one PCR reactions tested positive for the presence of the deletion bands of the correct sizes. We therefore conclude that the deletion-containing bands represent a true deletion in the targeted gene.

          The well that contains the deletion was identified by PCR using

20   DNA from the 96-well microtiter plate that was used to make pool 36. DNA from each row and column of the plate was prepared and tested by PCR. Triplicate and duplicate PCR reactions were performed for each row and column, respectively. A PCR using DNA from one indicted well amplified a band of the expected length in all reactions. Thus, we conclude that that well

25   contained the deletion.

Table I hop-1; sel-12 double mutant phenotypes.

Maternal Genotype	Zygotic Genotype	Glp-like Defects		Lin-12-like Defect
		Sterile	Mel	2AC††
hop-1/+;sel-12 *	hop-1;sel-12	yes§	N.A.¶	24/24
hop-1;sel-12/+ †	hop-1;sel-12	no	yes∏	0/25
hop-1/+;sel-12/+ ‡	hop-1;sel-12	no	yes**	N.D.‡‡

* The complete genotype is hop-1(nr2003)/unc-73(e936)dpy-5(e61);sel-12(nr2011).

† The complete genotype is either hop-1(nr2003);sel-12(nr2011)/pha-2(ad472)dpy-3(e27) or hop-1(nr2003);sel-12(nr2011)/egl-17(e1313)unc-1(e538).

‡ The complete genotype is hop-1(nr2003)/unc-73(e936)dpy-5(e61);sel-12(nr2011)/+.

§ non-Unc non-Dpy progeny (n=147) of hop-1/unc-73 dpy-5; sel-12 mothers were cloned as L4s and incubated for 24-36h. Of these, 60% were Egl and 40% were sterile. PCR analysis of 48 Egl and 44 sterile animals indicated that all of

the Egl animals were heterozygous for the *hop-1* deletion, whereas all of the sterile animals were *hop-1* homozygotes.

In a separate experiment, the germline phenotype of 59 young adult sterile animals was scored using DIC microscopy.

Each gonad arm contained 50-100 sperm, no oocytes, and no undifferentiated germ cells (see Figure 2), a phenotype

resembling that of *glp-1(lf)* mutants (22).

¶ N.A., not applicable

Π non-Dpy progeny (n=287) of *hop-1;sel-12/pha-2 dpy-3* mothers were cloned at the L4 and incubated for 24-36h. Of

these, 64% were wild type, 29% were filled with dead eggs (the Mel phenotype), 5% were Egl with live progeny and 2%

were sterile. PCR analysis of 20 wild type and 20 Mel animals indicated that all of the wild type animals were

heterozygous for the *sel-12* deletion, whereas all of the Mel animals were *sel-12* homozygotes. The Egl animals with

live progeny were all *sel-12* heterozygotes, indicating that in the absence of *hop-1*, a single wild-type copy of *sel-12* is

not always sufficient for normal egg laying. The rare sterile animals do not appear to have a germline proliferation

defect; this sterility has not been characterized further.

⁎⁎ Construction of the *hop-1/+;sel-12* strain generated animals of the genotype *hop-1/unc-73 dpy-5;sel-12/+* as

intermediates. Twenty Egl, non-Unc non-Dpy progeny of these animals were cloned; fourteen segregated both Glp

sterile and Unc Dpy animals (genotype *hop-1/unc-73 dpy-5;sel-12*), whereas six had a Mel phenotype, producing only

dead embryos. The Mel animals were genotyped by PCR and all of them were *hop-1; sel-12*.

†† The number of animals with two ACs and the total animals scored are indicated. Wild-type *C. elegans*

hermaphrodite gonads generate a single anchor cell, whereas *lin-12* null mutant gonads generate two (29). The

number of ACs in non-Unc non-Dpy progeny of *hop-1/unc-73 dpy-5; sel-12* mothers or non-Unc progeny of *hop-1;sel-*

12/egl-17 unc-1 mothers was determined during L3 or L4 using DIC microscopy (see Figure 2). After scoring, animals

were allowed to develop to adulthood. Adult *hop-1; sel-12* animals could be distinguished from their heterozygous

siblings based on their sterile or Mel phenotype.

‡‡ N.D., not determined.

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Table 2

List of primers used to screen for sel-12 deletions

Name	derives from	Sequence 5'-3'	pair with:	PCR product size
sel-12.F1	1738-1758	CATGTTCCATACTGGCAATCT	R1	3097 bp
sel-12.R1	4835-4815	TCCAGGTTTACTCCAGTCTTG	F1	3097 bp
sel-12.F2	1913-1931	GCTCAGGGTTGACACACTT	R2	2670 bp
sel-12.R2	4583-4563	TTACCTTTATTATTGGACGAA	F2	2670 bp

Table 3

List of PCR primers used to screen for *hop-1* deletions

Name	derives from	Sequence 5'-3'	pair with:	PCR product size
hop-1.F1	1991-2015	TCAAAGACCGATAATGTTAGTTGTA	R1	2968 bp
hop-1.R1	4958-4936	TTCCAGATATGCCAACTGAAGTG	F1	2968 bp
hop-1.F2	2098-2120	GCCGACCTGAAAGATAATGAAAT	R2	2709 bp
hop-1.R2	4806-4785	ATAAAGCCGAAGGTTAGACGAT	F2	2709 bp
hop-1.F3	2234-2256	TTGTTTGAAAATAGGTGGTTTGG	R3	1574 bp
hop-1.R3	3807-3787	TCTGTCTGGGAATCACTGGAG	F3	1574 bp

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TABLE 4

	Range	Preferred Range	% IVM [1] genomes
EMS (Ethyl methane sulfonate)	.05-2%	.25%-.5%	1.2-2%
ENU (Ethyl nitroso urea)	0.1-2.5mM	.4-6mM	1-1.5%
UV*/TMP (Trimethylpsoralen)	1500-20,000 uW x sec./cm²	8,000-12,000 uW x sec./cm²	.5-.75%
DEO (Di-epoxyoctane)	1-7.5mM	2-4mM	.5-.8%
DEB (Di-epoxybutane)	.05-.5mM	.05-.1mM	.8-1.3%

*UV dose is measured in microWatts per square centimeter times seconds of exposure.

Table 5: Akt
Primer List

Name	derives from	Sequence 5'-3'	#nt	GC	Tm	pair with:	PCR
R03E1.F1	8735-8756	GCAGCAAAAAAGGTCACATCCC	22	50	57	R1	5285
R03E1.R1	14019-13996	GTATGGCATGACAGATTTCCACAG	24	46	55	F1	5285
R03E1.F2	8752-8773	ATCCCCTCTCTTCGCTGCTATC	22	55	56	R2	4842
R03E1.R2	13593-13570	CAGGAAAGGAATGGAAATTAGCCC	24	46	58	F2	4842
R03E1.F3	8817-8839	CCGCTCAAGTTTTGTCTGCAATC	23	48	58	R3	3075
R03E1.R3	11891-11869	CGCCAGCATTATCACACTCTGTG	23	52	57	F3	3075
R03E1.F4	8879-8901	CACTAGGGGTGTTTGTTTTTCCC	23	48	56	R4	2937
R03E1.R4	11815-11794	AGAAAGGCGATGTTGTGAGGGC	22	55	59	F4	2937

What is claimed is:

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Claims

1. A nematode having a deletion in the *hop-1* gene, said deletion introduced by artifice.

2. The nematode of claim 1, said nematode further comprising a mutation in *sel-12*.

3. The nematode of claim 1, said nematode further comprising a mutation in *spe-4*.

4. A nematode having deletions in *hop-1*, *sel-12*, and *spe-4* at least one of said mutation introduced by artifice.

5. A method for identifying a compound which is capable of affecting the course of Alzheimer's disease, said method comprising exposing a nematode having a *hop-1* deletion and a second deletion in a gene selected from the group consisting of *sel-12* and *spe-4* to an effective amount of a compound, screening for an alteration in the phenotype of said nematode, an alteration in the phenotype of said nematode indicating a compound capable of affecting Alzheimer's disease.

6. The method of claim 5, wherein said second deletion is a deletion in *sel-12*, said phenotype is selected from the group consisting of dead eggs or sterility, and said alteration is an alteration in either the number viable eggs or fertile offspring where such eggs or offspring would otherwise be absent.

7. A method for identifying a gene which affects the course of

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Alzheimer's disease, said method comprising selecting a reversion mutation in

a nematode, said nematode having a *hop-1* deletion and a second deletion in a

gene selected from the group consisting of *sel-12* and *spe-4,* and screening for

an alteration in the phenotype of said nematode, an alteration in the phenotype

5   of said nematode indicating a compound capable of ameliorating Alzheimer's

disease.

8. The method of claim 7, wherein said second deletion is a deletion

in *sel-12*, said phenotype is selected from the group consisting of dead eggs or

sterility, and said alteration is an alteration either of viable eggs or fertile

10   offspring where such eggs or offspring would otherwise be absent.

9. The method of claim 7, wherein said method further comprises the

step of selecting for a suppressor of said alteration in said phenotype.

10. A method for identifying a gene which affects the course of

Alzheimer's disease, said method comprising screening for a mutation which

15   phenocopies the phenotype of a nematode having a mutations including *(hop-1;*

spe-4); (hop-1; sel-12) or *(hop-1; sel-12; spe-4).*

11. A method for identifying a compound which affects course of

Alzheimer's disease, said method comprising screening for a compound which

phenocopies the phenotype of a nematode having a mutations including *(hop-1;*

20   *spe-4); (hop-1; sel-12);* or *(hop-1; sel-12; spe-4).*

12. The method of claim 10 or 11, wherein said phenotype is the

Bag of Dead Eggs phenotype.

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13. An isolated nucleic acid encoding HOP-1, said nucleic acid having a deletion mutation.

14. The isolated nucleic acid of claim 12, wherein said nucleic acid is genomic DNA.

5   15. The isolated nucleic acid of claim 12, wherein said nucleic acid is RNA.

16. A method for constructing a nematode having a genetic deletion in a region of choice, said method comprising the steps of:

a) exposing said nematode to an appropriate mutagen;

10   b) propagating said nematode for at least one generation;

c) amplifying the nucleic acids of said nematode using at least two nucleic acid primers from the region of the genome where said genetic deletion is desirable; and

d) detecting the presence of a amplified nucleic acid which is shorter

15 than the amplified nucleic acid from a non-mutagenized nematode.

17. The method of claim 16, wherein said mutagen is trimethylpsoralen and said mutagen is administered following exposure to a second mutagen, said second mutagen being ultraviolet irradiation.

18. The method of claim 16, wherein said method further comprises

20 the steps of initially amplifying nucleic acid from a pool of nematodes said pool comprising at least 1000 genomes derived from distinct mutagenized nematodes.
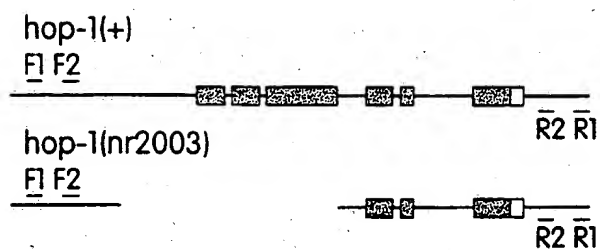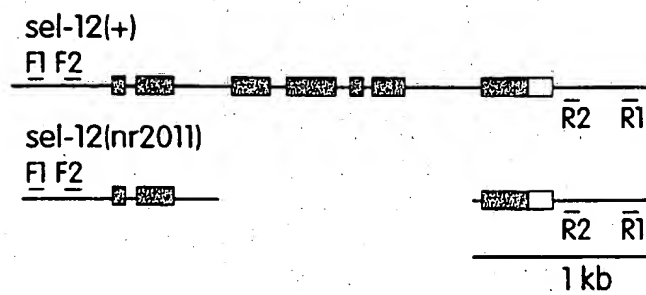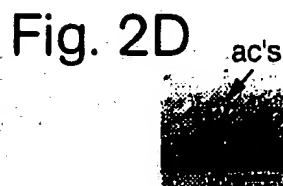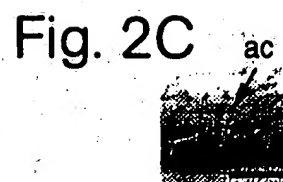
hop-1(+)
F1 F2

R2 R1

hop-1(nr2003)
F1 F2

R2 R1

Fig. 1A

sel-12(+)
F1 F2

R2   R1

sel-12(nr2011)
F1 F2

R2   R1

1 kb

Fig. 1B

Fig. 2A

Fig. 2B

Fig. 2C

Fig. 2D
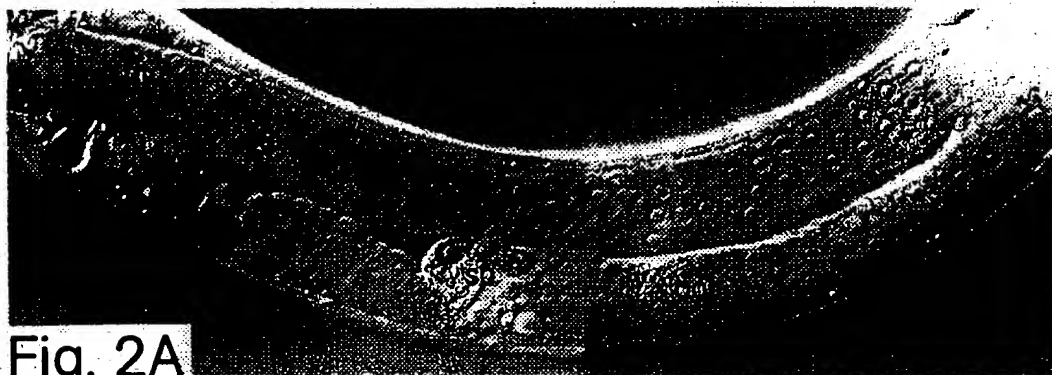
N2

Fig. 3A

glp-1

Fig. 3B

hop-1;sel-12

Fig. 3C

Fig. 3D

Fig. 3E

Fig. 3F
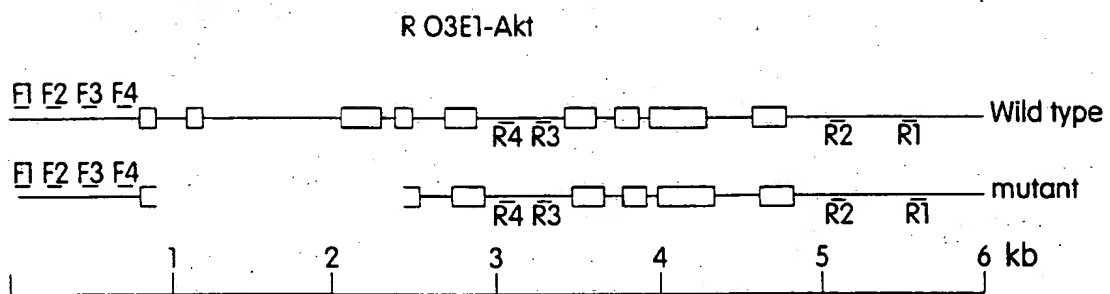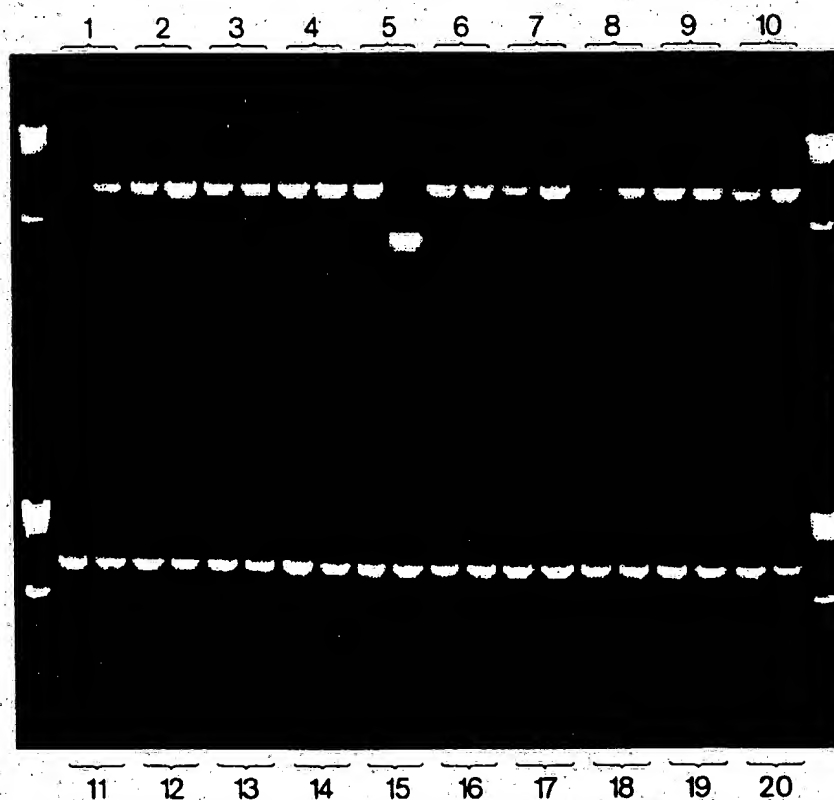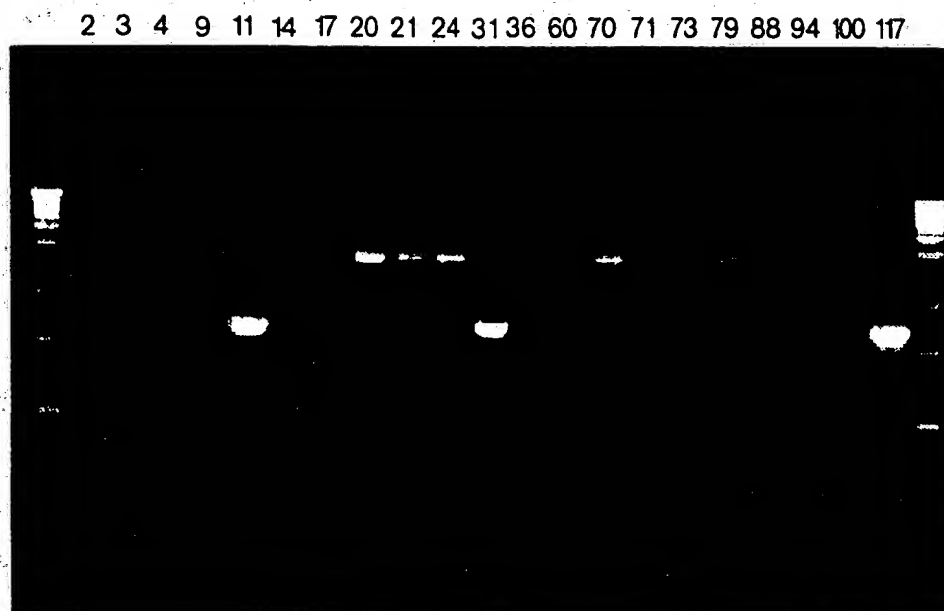
R O3E1-Akt

Fig. 4

Fig. 5

Fig. 6
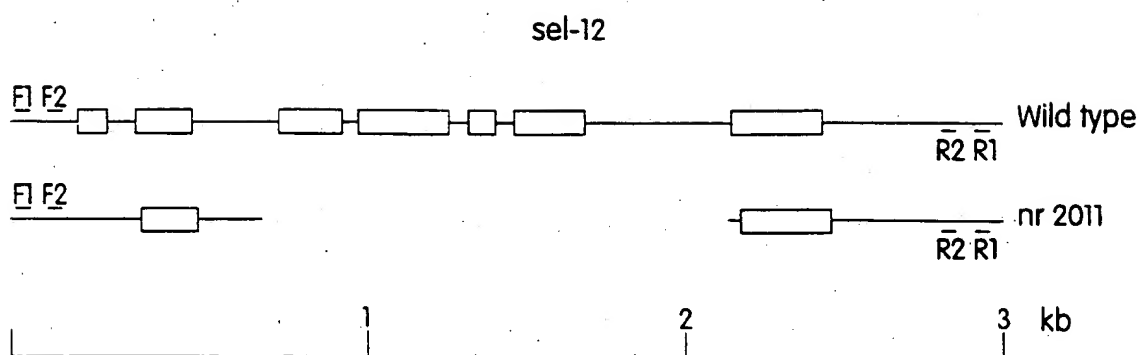
Fig. 7

sel-12 (+) bp 2525-2545 TCTATCAA T ATTTCTTTGGAA

sel-12 (+) bp 3955-3975 CATA T TTTTTTTAACAATAAT

sel-12 (nr2011) bp 2525-2545 TCTATCAATTTTTTTTAACAA

Fig. 8

hop-1

Fig. 9

```
                                      ↓   ↓
hop-1(+) bp 2300-2500  AAACCAC A CACTTTCTAGACA

                                          ↓   ↓
hop-1(+) bp 3490-3510  GCTACACAATAAA A GCCCAAA


hop-1(nr2003) bp 2300-2500  AAACCACAGCCCAAAATATAA
```
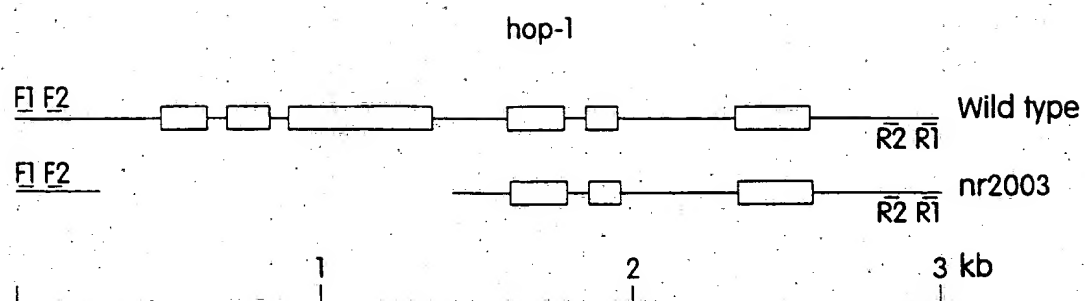
Fig. 10

WT hop-1/+;sel-12/+

Egl(Bag) hop-1/+;sel-12/sel-12(m$^+$/z$^+$;m$^+$z$^-$)

Egl (Bag) hop-1/hop-1;sel-12/sel-12 (m$^-$/z;m$^-$/z)

Dead egg hop-1/hop-1;sel-12/sel-12 (m$^-$/z$^-$;m$^-$z$^-$)
Glp-1 DEFECTS (E.G. EXTRA PHARYNX)?

Sterile (Glp) hop-1/hop-1;sel-12/sel-12 (m$^+$/z$^-$;m$^-$z$^-$)
Lin-12 DEFECTS (E.G. EXTRA ANCHOR CELLS)?

STERILE (Glp) hop-1/hop-1;sel-12/sel-12 (m$^+$/z;m$^-$z$^-$)

Egl(Bag) hop-1/+;sel-12/sel-12 (m$^+$/z$^+$;m$^-$z$^-$)
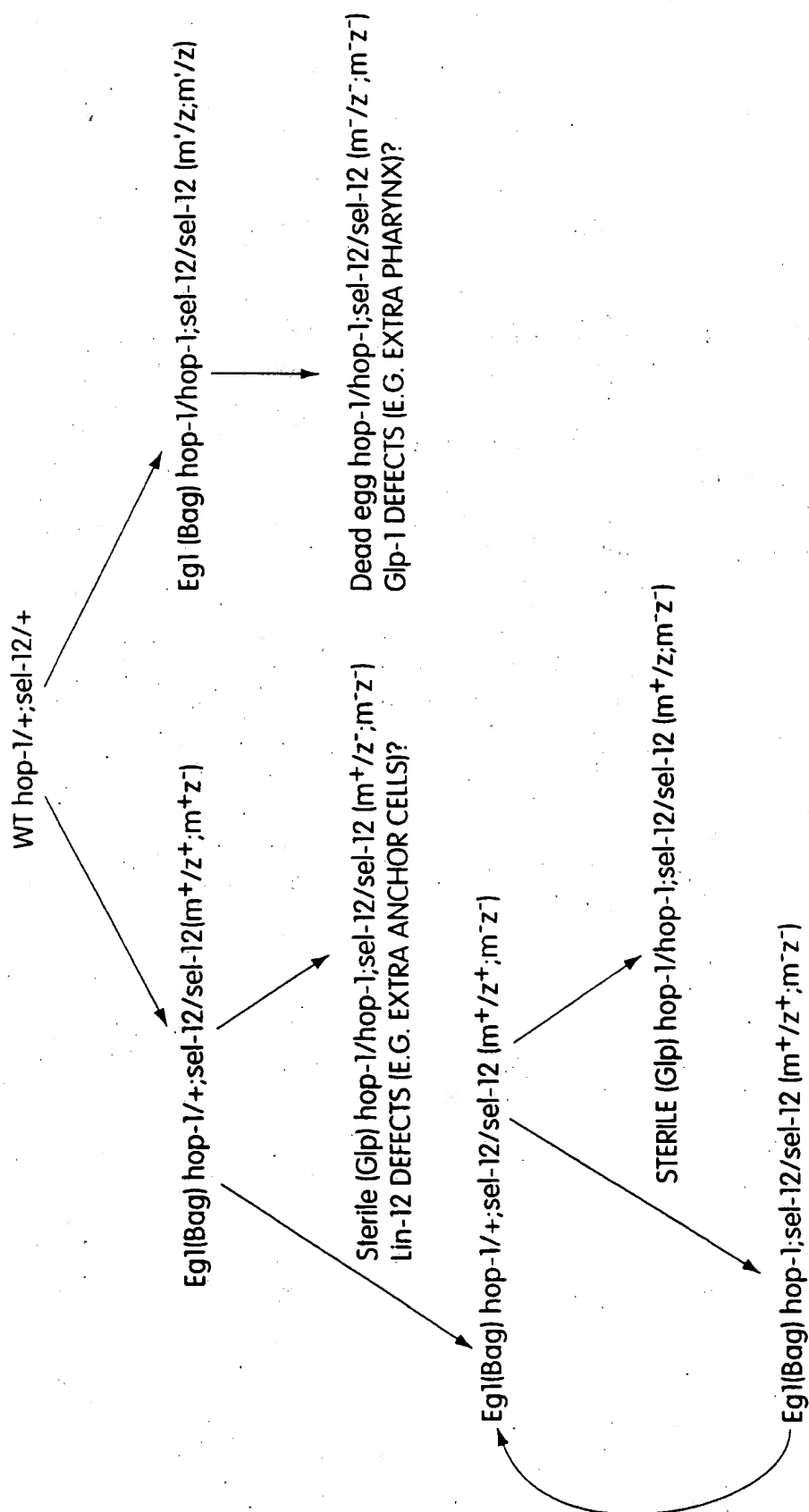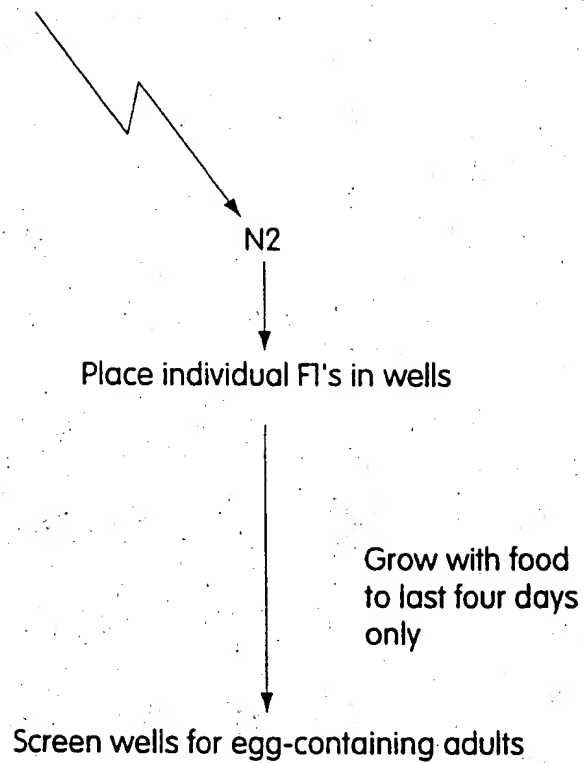
Egl(Bag) hop-1;sel-12/sel-12 (m$^+$/z$^+$;m$^-$z$^-$)

maternal contribution from neither: dead egg
maternal contribution from only psl: sterile (Glp)
maternal contrabution from only sel-12: ?
zygolic rescue of maternal defects?

Fig. 1

12/14

N2

↓

Place individual F1's in wells

Grow with food
to last four days
only

Screen wells for egg-containing adults

Validation

1. Verify sibs throw Bags of Dead Eggs
2. Test dead embryos for Notch defects
3. Test for suppression of lin-12 (D)

Fig. 12

FAD presenilin mutations are:

	Loss of function	Gain of funcction
Relevent Suppressor Mutations	Loss-of-function (LOF)	Gain-of-function (GOF)
Relevent Revertants of LOF Suppressor	GOF	LOF
Relevent Revertants of GOF Suppressor	LOF	GOF
Relevent Phenocopy Mutations	GOF	LOF

Fig. 13

Fig. 14

INTERNATIONAL SEARCH REPORT

A.   CLASSIFICATION OF SUBJECT MATTER

IPC(6)   :C12N 5/00, 15/00; A61K 49/00; C12Q 1/70

US CL   :800/8, 9, 12, 13; 424/9.2; 435/5, 172.3; 536/23.1

According to International Patent Classification (IPC) or to both national classification and IPC

B.   FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :   800/8, 9, 12, 13; 424/9.2; 435/5, 172.3; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CHEMICAL ABSTRACTS

C.   DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	L'HERNAULT, S.W. et al. Mutation of a Putative Sperm Membrane Protein in Caenorhabditis elegans Prevents Sperm Differentiation but Not Its Associated Meiotic Divisions. Journal of Cell Biology, October 1992, Vol. 119, No. 1, 55-68, especially pages 56 and 58-60.	1-12 and 16-18
Y	LEVITAN, D. et al. Assessment of Normal and Mutant Human Presenilin Function in Caenorhabditis elegans. Proceeding National Academy of Sciences (USA). December 1996, Vol. 93, pages 14940-14944, especially pages 14942-14944.	1-15

[X]   Further documents are listed in the continuation of Box C.   [ ]   See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"B"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search

05 AUGUST 1998

Date of mailing of the international search report

1 9 AUG 1998

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LEVITAN, D. et al. Facilitation of lin-12-Mediated Signalling by sel-12, a Caenorhabditis elegans S182 Alzheimer's Disease Gene. Nature. 28 September 1995, Vol. 377, pages 351-354, especially pages 352-354.	1-12 and 16-18
Y	NAITO, M et al. Identification of a Homeobox-Containing Gene Located Between lin-45 and unc-24 on Chromosome IV in the Nematode Caenorhabditis elegans. Nucleic Acids Research. 1992, Vol. 20, No. 12, 2967-2969, especially 2967.	16-18